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HETEROAUXIN AND THE PRODUCTION OF TETRAPLOID SHOOTS BY THE CALLUS METHOD IN *BRASSICA OLERACEA*

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(With Five Text-figures)

1. INTRODUCTION

GREENLEAF (1938) obtained in *Nicotiana* hybrids a relatively high percentage of tetraploid callus shoots by applying a 1 % paste of heteroauxin to the cut surfaces of decapitated seedlings. It was not, however, possible to show that this high percentage (13·6) of tetraploids obtained was due to the heteroauxin treatment, since the simple decapitation technique does not produce calluses with shoots in *Nicotiana*.

In *Brassica oleracea* (kales and cabbages) calluses with leafy shoots can be obtained by simple decapitation, but a higher percentage of shoot-bearing calluses is obtained if heteroauxin paste is applied to the cut surface (Goldberg, 1938; Howard, 1938). In this paper it is shown that in *Brassica oleracea* heteroauxin treatment does not increase the percentage of tetraploid callus shoots over that obtained by simple decapitation, and there is also given a short account of the origin of callus shoots.

2. *BRASSICA OLERACEA* RESULTS

Most of the seedlings used for obtaining callus shoots were kales (see Table 1). The seedlings were decapitated when they had two large leaves. The decapitation was done as high as possible up the stem. Axillary buds were also removed at decapitation and as they appeared each week after decapitation. The decapitated plants were placed under bell-jars in a shady place so as to keep the cut surface moist. If the cut surface is allowed to become very dry, no callus shoots are obtained.

The heteroauxin (indole-3-acetic acid) treatment consisted in the application of a 1 % paste of the hormone in anhydrous lanoline to the cut surface immediately after decapitation. An account of the effects of this treatment, including the production of adventitious roots, will be found in both Goldberg (1938) and Howard (1938).

The callus shoots were separated from the callus when they were 2–4 in. tall and placed in moist sand in either a cold frame or under bell-jars. To obtain good rooting of these cuttings was not difficult. The most important factor in rooting them appeared to be to make the cut near the base of a leaf.

The cuttings were grown on to flowering. Diploids and tetraploids were then recognized by measuring pollen grains. Pollen-grain size was found to be a very satisfactory method of recognizing tetraploids in these experiments and also in colchicine work with *Brassica*. All cuttings except one eventually flowered. Some cuttings, however, missed flowering in their first two years, and it is this fact which accounts for this paper being written in 1941 and not in 1939. The tetraploids were confirmed by cytological examination and an account of their cytology has been published (Howard, 1939).

The results are given in Table 1. It will be seen that only three calluses produced tetraploid shoots. Nevertheless, the results do show that in *Brassica oleracea* heteroauxin treatment does not produce any striking increase in the percentage of tetraploid shoots obtained.

Table 1. *Frequency of tetraploid shoots from* Brassica oleracea *calluses*

Variety	Exp. nos.	Treatment	No. of plants de-capitated	No. of diploid cuttings	No. of tetra-ploid cuttings	% tetra-ploid
Cabbage	III, XII, XV	Decapitation only	35	74	0	0
1000-H Kale	XVII	Decapitation only	30	80	3*	3·7
1000-H Kale	XIX	Decapitation only	30	133	0	0
1000-H Kale	XXI	Decapitation only	10	28	0	0
	Total	Decapitation only	105	315	3	0·97
Marrow S. Kale	XXVII	1% paste heteroauxin	12	73	0	0
1000-H Kale	XXXI	1% paste heteroauxin	15	76	2†	2·6
	Total	1% paste heteroauxin	27	149	2	1·3

* All three cuttings (one of them was a diploid-tetraploid sectorial chimaera) came from the same callus, XVII 24; there were also seven diploid cuttings from this callus.

† Two cuttings from separate calluses, XXXI 7, seven diploid plus one tetraploid cuttings and XXXI 10, one diploid plus one tetraploid cutting. The tetraploid from XXXI 7 did not flower and its chromosome number was determined from root-tip counts.

3. Results in other plants

The percentages of tetraploid callus shoots obtained by other workers for various plants is given in Table 2. The results of Lindstrom & Koos appear to be exceptional. The *Brassica* results are similar to those shown in Table 1. It also appears that in *Lycopersicon esculentum* the frequency

of tetraploid shoots is about 6 % as compared with under 1 % in *Brassica* species. The 13·6 % of tetraploids obtained by Greenleaf might therefore be characteristic of *Nicotiana* and not due to the heteroauxin treatment.

Table 2. *Frequency of tetraploid shoots obtained by previous workers*

Species	Reference	Treatment other than decapitation	Total no. of cuttings	No. of tetra-ploid cuttings	% tetra-ploid
Lycopersicon esculentum	Jørgensen (1928)	None	278	16	5·8
Lycopersicon esculentum	Jørgensen (1928)	None	147	9	6·4
Solanum nigrum × *luteum* F_1	Jørgensen (1928)	None	342	7	2·0
Lycopersicon esculentum	Sansome (1930)	None	—	—	About 6
Lycopersicon esculentum	Lindstrom & Koos (1931)	Petrolatum	309	109	35·3
Brassica oleracea tetraploid × *carinata* F_1	Karpechenko (1937)	None	455	3	0·66
Brassica oleracea tetraploid × *chinensis* F_1	Karpechenko & Bogdanova (1937)	None	186	1	0·54
Nicotiana hybrids	Greenleaf (1938)	1 % heteroauxin	1973	270	13·6

4. THE ORIGIN OF CALLUS SHOOTS

Mather (1933) has given an account of the cytology of the origin of callus shoots in the tomato (*Lycopersicon esculentum*). He does not, however, describe the actual origin of the callus but only discusses the cytology of cells in the various parts of the callus. It is also extremely difficult to understand the plane in which the section drawn as Fig. 1 in his paper was cut. This is presumably due to the fact that Mather cut the calluses into small pieces for fixation.

Externally, tomato and *Brassica* calluses differ greatly in their development. In tomatoes the callus bulges out from the cut surface while in *Brassica* the cut surface remains flat and becomes slightly corky. Later in tomatoes shoots appear as bulges on the callus while in *Brassica* the shoot buds are first seen as small, usually purplish, tubercles pushing their way through the flat callus surface. Decapitated *Brassica* plants treated with heteroauxin produce bulgy calluses which resemble to a certain extent tomato calluses produced by decapitation alone.

Longitudinal sections through both tomato and *Brassica* calluses of different ages (1, 2 and 4 weeks after decapitation) were examined to ascertain the origin of calluses and callus buds. The short account of calluses given below agrees entirely with the very full accounts given by Priestley & Swingle (1929).

The first stage in the formation of a *Brassica* callus is shown in Fig. 1. The layer of cells, two or three cells down from the cut surface, becomes meristematic and divides by walls parallel to the cut surface.

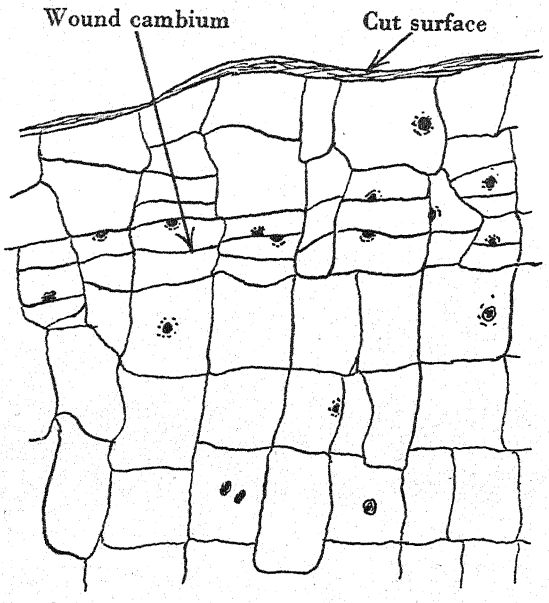

Fig. 1. Section through kale callus, 1 week after decapitation.

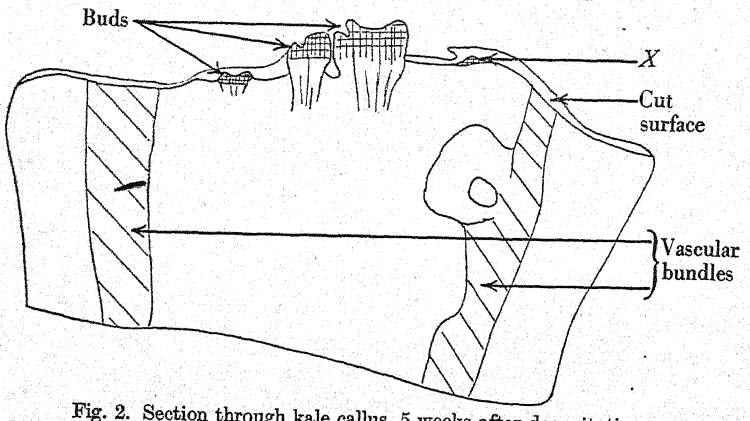

Fig. 2. Section through kale callus, 5 weeks after decapitation.

It is this layer of cells which becomes corky and protects the cut surface of the shoot. This layer can also be seen in Fig. 3, which is a section through an older kale callus. The meristematic tissue which will give rise to the callus buds also arises from this layer—this is shown quite well in

Fig. 3. The meristematic small-celled tissue is seen in Fig. 3 to be beginning to grow upwards and to be differentiating into a bud. The whole section (see Fig. 2) includes three recognizable buds in addition to the meristem which is marked *X*. As one removes shoots from a callus new buds are formed. These new buds are presumably formed from meristems like that marked *X* in Fig. 2. It will be noticed in Fig. 3 that the cells below the meristem have also divided.

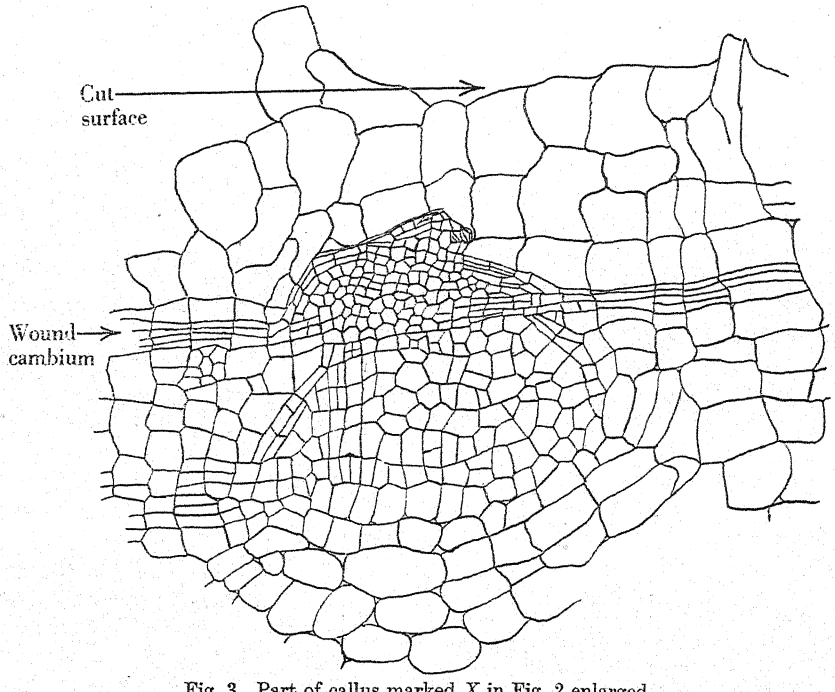

Fig. 3. Part of callus marked *X* in Fig. 2 enlarged.

Figs. 4 and 5 show a section through a one-week-old callus of a tomato. The cut surface of this callus had been kept moist by placing a bell-jar over the plant. It will be seen from Fig. 5 that no continuous wound cambium like that found in the *Brassica* calluses has been produced. Instead, there are two regions of small meristematic cells situated over the internal phloem of the vascular bundles. These meristematic regions grow rapidly and the adjacent tissues also become meristematic. It is the growth of such regions which produces the bulges of the callus.

In the sections of both the *Brassica* and tomato decapitated stems it can be seen that the cells, which divide to form the new meristematic tissues from which the callus buds later originate, are large vacuolated

ones. This is an important fact to be taken into account in considering the origin of tetraploid areas in these calluses. Figs. 1, 3 and 5 all illustrate this point. It can be seen that the cells adjacent to those which have produced the meristematic cells are large and vacuolated.

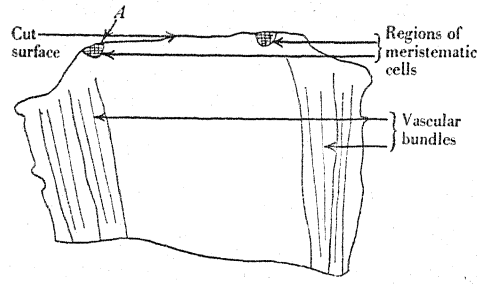

Fig. 4. Section through tomato callus, 1 week after decapitation.

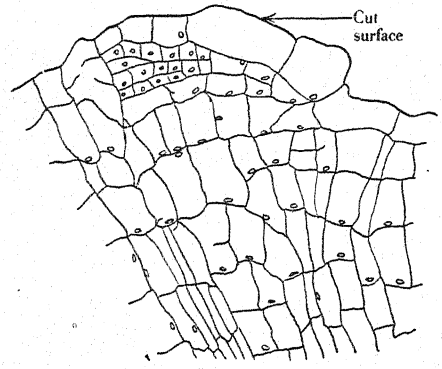

Fig. 5. Part of callus marked *A* in Fig. 4 enlarged.

5. Hypotheses to account for the occurrence of tetraploid callus shoots

It is possible to suggest four reasons why tetraploid callus shoots should be produced: (*a*) the occurrence of binucleate cells in stems, (*b*) the occurrence of tetraploid nuclei in some cells of the stem before decapitation, (*c*) the occasional failure of cell-wall formation when vacuolated cells divide, and (*d*) a breakdown in the normal course of cell division other than the failure of cell wall formation during the division of vacuolated cells.

Jørgensen (1928) supports a suggestion originally made by Winkler that the presence of binucleate cells in the stems before decapitation will account for the production of tetraploid shoots by calluses, and he refers to a paper by Beer & Arber (1919) on the occurrence of binucleate cells

in the pith and cortex especially of many plants. It is interesting to note that Beer & Arber did observe binucleate cells in *Brassica oleracea*. It is suggested that the two nuclei in the one cell divide at the same time and unite to form a single metaphase plate which will be tetraploid.

The second explanation—that tetraploid callus shoots are due to the presence of tetraploid nuclei in the stems before decapitation—appears to be highly probable. Thus Levan (1939) has shown that the old vacuolated cells in *Allium* roots contain diplochromosomes and that, when such cells are stimulated to divide by the application of heteroauxin, tetraploid metaphase plates are formed. The heteroauxin does not produce the tetraploid cells but only stimulates the old vacuolated cells which are already tetraploid to divide. Tetraploid cells are not produced in the young cells at the root tip. Similarly, Dermen (1941) found that polyploid cells were produced only in differentiated parenchyma tissues and not in the cambial regions of bean (*Phaseolus*) stems treated with naphthalene-acetic acid. Diplochromosomes and the subsequent formation of tetraploid metaphase plates in which the chromosomes are paired have been observed in many plants, e.g. *Spinacia* and *Cucumis* (references in Barber, 1940), in the nodules of Leguminosae (see Dermen, 1941), and in *Iberis semperflorens* (Figs. 14–16 of Manton, 1935). Since the meristems which ultimately give rise to callus buds are formed by the division of old vacuolated cells, the presence of tetraploid callus shoots would be expected from the results of Levan & Dermen. The differences in percentage tetraploids from calluses of *Brassica* and tomato have, however, still to be explained. This problem is considered in the last section of this paper.

The third explanation—that somatic doubling occurs in some cells of a callus because of occasional failure of cell-wall formation when large vacuolated cells divide—was suggested by Mather (1933). He gives a number of figures showing cell divisions in large vacuolated cells and others showing binucleate cells. Mather did not ascertain the precise stage at which nuclear fusion takes place in the binucleate cells but agrees with the suggestion of Lindstrom & Koos that it may well occur at several points in the nuclear cycle. While it is quite reasonable to suggest that cell-wall formation may occasionally fail when vacuolated cells divide, it is important to note that Sinnot & Bloch (1941) have shown that there is a regular mechanism by means of which division of the nucleus in vacuolated cells is followed by cell-wall formation. It seems unlikely that this mechanism would break down in 6% of the cells in the tomato calluses.

Fourthly, tetraploid cells might be produced in callus formation by a breakdown in the normal processes of cell division other than failure of cell-wall formation, e.g. a breakdown in anaphase separation followed by the formation of a single tetraploid resting nucleus. Such a breakdown might be due either to the cells being vacuolated or to the effect of wound hormones.

While it is not possible to decide conclusively what is the reason for the occurrence of tetraploid callus shoots, the second explanation appears to be the most probable. It ought, however, to be possible to observe the first divisions of the vacuolated cells in decapitated tomato stems and ascertain if tetraploid metaphases with paired chromosomes occur.

6. Heteroauxin and the production of tetraploids

It has previously been mentioned that the work of Levan (1939) showed that heteroauxin did not produce tetraploid cells but only stimulated cells which were already tetraploid to divide. We should not, therefore, expect to obtain a higher percentage of tetraploid shoots from heteroauxin treatment than from calluses obtained by decapitation only. This was found to be true for *Brassica oleracea*.

Assuming that tetraploid cells in calluses are produced by the division of vacuolated cells which contain nuclei with diplochromosomes, it would appear from the percentages of tetraploid callus shoots obtained that less than 1 % of the vacuolated cells in kale stems contain nuclei with diplochromosomes, while the corresponding frequencies in tomato and *Nicotiana* are 6 and 13 % respectively. An alternative explanation of the low frequency of tetraploids obtained in *Brassica* is that diploid cells might grow much faster than tetraploids in the calluses. Observations of diploid-tetraploid chimaeras produced by colchicine treatment in *Brassica* do not support this suggestion.

7. Summary

1. In *Brassica oleracea* the frequency of tetraploid callus shoots obtained by decapitation only was found to be about 1 %. The frequency from calluses obtained by heteroauxin treatment was also about 1 %.

2. The origin of new meristems from vacuolated cells in calluses is described.

3. Theories to account for the occurrence of tetraploid callus shoots are considered. It is suggested that the tetraploid areas in calluses are caused by the division of vacuolated cells which contain nuclei with diplochromosomes.

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SOME EFFECTS OF THE DRUG COLCHICINE ON CELL DIVISION

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(With Plate 1 and Three Text-figures)

Introduction

The investigations which form the basis of the following paper were begun in 1938. At this time, although it was known that colchicine treatment caused doubling of the chromosome number the exact mechanism had not been fully worked out. During the course of the author's experiments on the mechanism of colchicine action Levan (1938, 1939*a*, 1939*b*, 1939*c*) and several other workers, namely, Mangenot (1938), Kostoff (1938), Nebel & Ruttle (1938) and O'Mara (1939) published papers along some of the lines on which the writer was working.

It will not be necessary in this account therefore to give exact descriptions of the course of mitosis under the action of colchicine, since this has already been dealt with by Levan and others.

I shall confine myself to dealing with those aspects which other investigators have not touched upon, showing that the results obtained do not in certain cases coincide with those of Levan.

Material and methods

Most of the work was carried out with *Allium Cepa* seedlings germinated on damp filter paper in Petri dishes. *A. zebdanense* and *Brassica nigra* were also used in certain experiments.

Fixations were made in La Cour 2BE and other osmic type fixatives. Root-tip divisions were examined in both transverse and longitudinal sections 20–25 μ thick.

Results

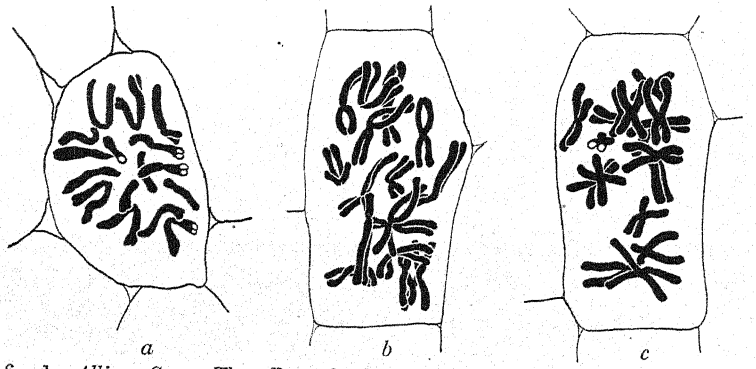
(1) *Effective concentrations*

It was first necessary to find out the most effective concentrations of colchicine and the length of time needed to bring about the characteristic chromosome doubling. Treatment with concentrations of $M/10{,}000$ (0·004 %) or less produced no effects with up to 4 hr. treatment, whilst $M/100$ (0·4 %) produced very marked effects. $M/1000$ (0·04 %) proved

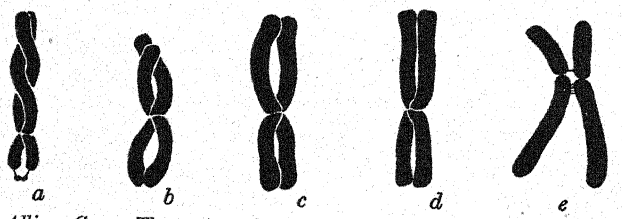
to be a very useful concentration for the plants under consideration. Treatment for 6, 12, 24, and 48 hr. and fixation 24 and 48 hr. after treatment formed the basis for the study of the cytological action of the drug.

(2) *Cytological action*

This has already been dealt with by Levan (1938). The observations made during the present research need therefore be only briefly summarized.



Text-fig. 1. *Allium Cepa.* The effect of colchicine on root mitoses. *a*, normal diploid metaphase plate; *b*, colchicine metaphase plate in an early stage; the chromatids are just beginning to separate; *c*, colchicine metaphase plate at a later stage in which the chromatids are quite widely separated. ×1150.

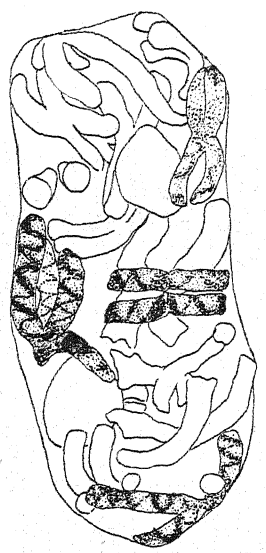


Text-fig. 2. *Allium Cepa.* The action of colchicine on different *c*-pairs. *a*, normal; *b*, *c*, *d*, chromatids slightly separated; *e*, chromatids widely separated. ×2500.

Colchicine appears to exert no influence upon resting nuclei or on mitotic prophase stages. It acts upon metaphase, anaphase and telophase by completely inhibiting the spindle mechanism. On the initiation of metaphase the nuclear membrane disappears and the chromosomes become scattered through the cell in a completely irregular manner. Relational coiling between each pair of chromatids gradually disappears, and although still joined together at the centromere they repel each other, producing an X-shaped or cruciform structure (see Text-figs. 1, 2). If the drug is applied to dividing cells that have reached anaphase or

telophase the chromosomes remain in the position to which they have moved. Even at telophase the spindle completely disappears under the influence of the drug. The absence of a spindle means that no cell wall is laid down, and at the next division twice the normal number of chromosomes will be present.

The X-shaped chromatid pairs were designated '*c*-pairs' or 'colchicine pairs' by Levan. He considered that the division of the centromere takes place in the course of a few hours after the beginning of the colchicine metaphase. The writer's own observations indicated, however, that the centromeres may not necessarily split until the resting stage



Text-fig. 3. *Allium Cepa*. Diagram of early restitution stage corresponding to telophase in the normal cell, showing the haphazard arrangement of the sixteen *c*-pairs (only four represented in detail). × 2500.

has been entered. The *c*-pairs become included in one nucleus which I have called in the following account the 'restitution nucleus'. Even in the early resting stage one can still observe the chromatids joined at the centromeres. At this stage in some cells spiralization can be plainly seen in some chromatids (see Text-fig. 3 and Pl. 1, fig. 1). In a small proportion of cells and especially with some of the longer chromosomes the centromeres split before the cell enters the resting stage, but this is not at all common. When the second division under the influence of the drug is initiated all the *c*-pairs seem to have separated. It seems therefore that in the majority of cases the centromere splits in the resting stage or early prophase of the following division.

A feature of the restitution nuclei is their irregularity, lobing and often fragmentation, indicative of the fact that the scattered chromosomes have not become aggregated together when passing into the resting stage. In root tips that have been subjected to the action of colchicine the number of cells that have undergone at least one division under the influence of the drug can be estimated by counting the number of this type of nucleus.

(3) *Recovery*

Seedlings of *Allium Cepa* were treated for 6, 12, 24 and 48 hr. in $M/1000$ colchicine solution and fixed 24 and 48 hr. after. It was seen that with the lighter doses (6, 12, 24 hr.), after leaving for 48 hr. before fixation, the spindle mechanism which was at first inhibited by the action of the drug showed more or less complete recovery of action. For 48 hr. doses about 50 % recovery was noted after 48 hr. interval. Thus the effect of the drug, if not administered in too great quantity, wears off in time and the cells again begin to divide normally. During this time they may have undergone one or more colchicine mitoses with a consequent doubling or quadrupling of the chromosome complement. When fixing after 24 hr. instead of 48 hr. there is a much smaller degree of recovery noted. In the early recovery stages one often finds multipolar spindles or several distinct spindles in one cell when the number of chromosomes per cell is fairly large.

Roots treated with $M/1000$ solution for 12 hr. exhibited a few tetraploid cells, whilst the roots which were given 24 hr. treatment showed quite a lot of tetraploid cells. The 48 hr. treatment showed quite large numbers of tetraploid and possibly octoploid cells. All the treated seedlings from this experiment which were allowed to grow produced tetraploid plants and gave eight quadrivalents at meiosis. Some of the older roots were, however, of a chimaeral nature with partly diploid and partly tetraploid tissues.

(4) *Rate of division*

A later experiment was designed to make a more accurate survey of the rate of division under colchicine treatment. Seedlings of *Allium Cepa* were gradually cooled in a thermos flask in a cool chamber to about 0° C. in order to stop all cell divisions and reduce the nuclei to the resting stage. The function of the thermos flask was to prevent too rapid cooling and enable all the dividing cells to pass to the resting stage. Starting the experiment with all the cells in the resting stage would ensure that

all the cell divisions occurring during subsequent colchicine treatment should have been under the influence of the drug during their whole course. Seedlings were treated for 1, 2, 3, 12, 24, 30, 33, 36, 39, 42, 45, 48, 54, 60 and 72 hr. and fixed at once. A similar series of controls was also put up. The numbers of prophases, meta- and anaphases, and telophases were counted for each root tip in the series of controls. In the treated roots fixed from 1 to 3 hr. after the commencement of treatment where both colchicine and normal divisions were taking place the typical colchicine metaphases were included with normal metaphases for counting purposes. In a similar way telophases and early restitution stages were grouped together; after 3 hr. no telophases were present.

The results are given in Table 1. The figures in subcolumn *a* in every case indicate the number of cells in the particular stage of division

Table 1

	Prophase		Normal meta- and anaphase. Diploid *c*-metaphase		Tetraploid *c*-metaphase		Telophase. Early restitution nuclei		Total no. of dividing cells
Treatment	*a*	*b* %	*a*	*b* %	*a*	*b* %	*a*	*b* %	
1 hr.: Treated	281	59	174	36·6	—	—	21	4·4	476
Control	300	60	172	34·4	—	—	28	5·6	500
2 hr.: Treated	307	61·5	183	36·7	—	—	9	1·8	499
Control	397	64·2	190	30·7	—	—	31	5·1	618
3 hr.: Treated	368	49·2	307	41	—	—	73	9·8	748
Control	418	63	201	30·3	—	—	45	6·7	664
12 hr. treated	467	33·4	454	32·4	—	—	478	34·2	1399
24 hr. treated	354	32·2	538	48·9	—	—	207	18·9	1099
30 hr. treated	No counts		No counts		1	—	No counts		—
33 hr. treated	353	22·8	504	32·6	19	1·2	673	43·4	1549
36 hr. treated	132	11·3	272	23·3	182	15·6	581	49·6	1167
42 hr. treated	234	22·8	265	25·9	166	16·2	359	35·1	1024
45 hr. treated	94	9·3	106	10·5	281	27·9	528	52·3	1009
54 hr. treated	123	13·7	66	7·3	237	26·3	474	52·7	900

referred to at the top of the column; the counts are made each from one root tip by the examination of serial sections. Subcolumn *b* expresses the counts as a percentage of the total number of cells undergoing division. The main points of interest brought out by the table are as follows:

The control seedlings show a fairly constant percentage ratio of prophases to meta- and anaphases (60 : 34·4; 64·2 : 30·7; 63·0 : 30·3), whilst the number of telophases is also fairly constant. Examination of the controls for the other times (the counts are not shown in the table) shows that this ratio remains roughly constant throughout the experiment. For the first 2 hr. the treated seedlings do not differ significantly from the controls. At the end of the third hour, however, the number of

colchicine metaphases has risen slightly. At the end of 12 hr. it has risen very considerably and the values for all three columns are now more or less equal. After 24 hr. the number of colchicine metaphases is about 1½ times the number of prophases. After 33 hr. the early restitution stages are very high in number. For the purposes of the table all those cells whose *c*-metaphase chromosomes are losing their discrete outline and are passing back into the resting stage have been classed as early restitution stage.

At 30 hr. the first tetraploid colchicine metaphase is visible, whilst at 33 hr. nineteen are quite easily seen. From 36 to 54 hr. treatment the number of tetraploid *c*-metaphases rises considerably. This is due to the fact that more and more cells have completed their first division under the influence of the drug and are starting on the second one.

From 45 to 54 hr. treatment there is a very sharp decline in the number of diploid *c*-metaphases. Reference to Table 1 will also show that after about 33 hr. treatment the number of prophases declines both absolutely and relatively to the number of other divisional stages.

A significant fact is the very high proportion of early restitution nuclei after the 33 hr. stage. From 36 hr. onwards they form about half of the total number of divisional stages in the colchicined root tip.

At the 54 hr. stage practically every resting nucleus is highly lobed, indicating that all the cells have passed through at least one colchicine division. A computation of the total number of cells dividing at the different times of sampling shows that this rises to the highest level at 12 hr. from the commencement of treatment (the total number of cells per root tip being approximately the same in all the samples taken). It is maintained at this level until 45 hr. after the commencement of treatment, after which it falls slightly (see Table 1). Between these two times the total number of dividing cells is about twice the number in an untreated root. This gives us a very rough guide to the delaying action of colchicine on the division cycle; the process of cell (or rather chromosome) division is about twice as long under the influence of the drug. This of course only applies to *Allium Cepa* root tips germinating on moist filter paper at room temperature and in fairly darkened conditions.

Seedlings treated for 60 and 72 hr. showed a decrease in the quality of fixation and a morbidity in the state of the cells.

The general points brought out in the experiment are that the meta-, ana- and telophases as represented by the blocked colchicine metaphases and early restitution stages are lengthened under the action of the drug so that the proportion visible at any one time goes up greatly. As has

been mentioned above the process of the division cycle is lengthened to about twice as much as in the normal cell. As one cycle of division under the influence of colchicine takes roughly 33 hr. (when the tetraploid divisions are beginning) the normal division cycle under these conditions but in the absence of colchicine would be expected to last approximately $16\frac{1}{2}$ hr.

(5) *Action of colchicine on growth—macroscopic effects*

A well-marked macroscopic effect of colchicine action on roots is the characteristic swelling just behind the root tip and the general increase in thickness of root and cotyledon with no corresponding increase in length (see Pl. 1, fig. 2). Levan considered this subterminal root swelling to be due to the increase in cell volume consequent upon the greater quantity of chromosomes present after colchicine doubling. 'The same cells which at the beginning contain 16 chromosomes have been adapted to house 500 or 1000 chromosomes' (Levan, 1938, p. 481). The results of the present author do not accord with this hypothesis.

In the first place the cells behind the root tip would not have passed through a mitotic division cycle under the influence of the drug, since the swollen zone involves cells farther back than the region where cell division occurs. Under the influence of colchicine, as we have seen above, the cells themselves do not divide, so that there is no possibility of those cells which are now behind the root tip having been formed during treatment. The cells in the swollen zone are maturing cells and would have the ordinary diploid chromosome number. Consequently the size (volume) should not be greater than in untreated material unless colchicine exerts an influence on the cell volume other than by increasing the number of chromosomes.

The following experiment was performed in order to obtain a rough guide to which cells in the normal root the colchicine swollen cells corresponded. At the beginning of the experiment roots were marked with Indian ink at 1 mm. intervals. One batch was then treated with colchicine whilst the other was left as a control. The cells which swelled in the treated roots were at the same distance from the root tip as those which elongated in the controls. It would seem from this experiment that the cells which normally increase in length parallel to the root axis may merely increase in all directions simultaneously, due possibly to some loss of polarity. It was therefore necessary to ascertain whether the swelling was merely due to an alteration of polarity or whether the

swollen colchicined cells were actually larger than the elongated cells of the normal root.

This was effected by measuring the average cell volume of a swollen portion of the cortical tissue in a typical colchicine-treated root and comparing it with the cell volume in a corresponding elongated portion of untreated root. A root that had been treated for 4 hr. with $M/100$ colchicine solution was fixed after 10 days when the swelling had reached a maximum; longitudinal sections were then cut 20μ in thickness. The method employed was to measure an area of the root cortex; by counting the number of cells in the area the average for one cell was calculated. To obtain the third dimension the width of the area was taken and divided by the number of longitudinal rows of cells passing through it. Similar measurements were made from an equivalent portion of cortex from a normal untreated root about 1 cm. behind the tip where the cells had undergone the customary increase in length. The measurements are shown in Table 2.

Table 2

	Colchicine treated	Untreated
Length of area measured	1·021 mm.	1·875 mm.
Breadth of area measured	0·447 mm.	0·2685 mm.
Area	0·456 sq. mm.	0·503 sq. mm.
Number of cells in area	65 (approx.)	47 (approx.)
Average cell area	$7{\cdot}01 \times 10^{-3}$ sq. mm.	$11{\cdot}4 \times 10^{-3}$ sq. mm.
Average number of cell rows	6	6·5
Average width of each cell	$0{\cdot}447/6 = 7{\cdot}45 \times 10^{-2}$ mm.	$0{\cdot}2685/6{\cdot}5 = 4{\cdot}13 \times 10^{-2}$ mm.
Average cell volume	$7{\cdot}01 \times 10^{-3} \times 7{\cdot}45 \times 10^{-2}$ $= 5{\cdot}2 \times 10^{-4}$ cu. mm.	$11{\cdot}4 \times 10^{-3} \times 4{\cdot}13 \times 10^{-2}$ $= 4{\cdot}7 \times 10^{-4}$ cu. mm.

This method is somewhat approximate in that it assumes the cells to be rectangular prisms. Nevertheless, it is of value in showing that the cells are of comparable sizes in the two tissues. In the case of the treated cells the average volume is $5{\cdot}2 \times 10^{-4}$ cu. mm., whilst the untreated cells are $4{\cdot}7 \times 10^{-4}$ cu. mm. Allowing for inaccuracies in the method a remarkably close agreement is found, the cells being of the same size order in both cases. This experiment was confirmed by treating the unopened flower buds of *Allium zebdanense* with colchicine. The filaments, style and petals all remained short and squat, showing that cell enlargement had taken place in all three directions instead of in the longitudinal one as is normally the case.

Although from the above experiments there is an indication that the polarity of the cells is destroyed under the action of colchicine it is still not quite clear whether this is a primary or only a secondary effect.

Czaja's (1935) results on the application of growth substances (heteroauxin) to germinating maize seem of interest here. He found that lateral application of heteroauxin to *Avena* roots and coleoptiles produced a swelling similar to that which is obtained by colchicine treatment. The transverse stretching of the cell walls he considered to be due to the fact that the heteroauxin was applied laterally and he concluded that cell-wall enlargement was controlled by the direction of transport of growth substances. Later workers such as Thimann (1936) have discounted Czaja's polarity theory, and it seems probable that he took too mechanistic a view of the processes involved. Levan (1939*b*), for instance, showed that the subterminal root swelling obtained by colchicine treatment could be mirrored by treating roots with growth substances not unilaterally but by total immersion. The general effect was one of inhibiting elongation and promoting swelling of the subterminal portion of the root tip.

Levan's work in this connexion, however, seems to be slightly contradictory. After noting that an increase in cell volume takes place in the cortical cells after treatment with growth substance (an explanation which we have just shown to be untrue when using colchicine) he goes on to state (p. 89): 'on the other hand it will be seen from the longitudinal section that a larger number of cell rows is present than normally. This is due to the fact that the meristematic cortical cells under the influence of growth substances start numerous mitoses.' An examination of his excellent plate (Fig. 1*a*) does not reveal this increase in cell rows. On each side of the stele there are 11–12 rows both in the swollen and unswollen regions. Later, in the same paragraph, he admits that extra cell divisions are not a necessary prerequisite for the subsequent swellings of the cells. It would seem, therefore, that the same processes take place here as with colchicine treatment. The cells probably do not increase in volume as compared with the elongated cells in the cortex of a normal root. The swelling may quite well therefore be due to a lack of polarity and not to increase in the number of cells or their individual volumes. The cell divisions that take place must be due to some other effect of the growth substances used and are obviously not directly connected with the root swellings which can equally well take place in their absence.

In elucidating the problem of the action of colchicine and of growth substances such as heteroauxin some attempt was made in the present experiments to discover whether the drugs exerted a direct effect on the lateral swelling of the cortical cells or whether the effect was a secondary

one due to the influence of the root tip. To try and test this the following experiments were set up:

Each of four batches of seedlings (eight per batch) received different treatment as follows:

(1) Root tips removed: treated with $M/100$ colchicine for 48 hr.
(2) Root tips removed: no treatment.
(3) No removal of root tips: treated with $M/100$ colchicine for 48 hr.
(4) No removal of root tips: no treatment.

No swellings were observed in batches (1), (2) and (4). Only those treated seedlings with intact root tips showed swellings. From this one must conclude therefore that the root swellings produced under the influence of colchicine are dependent upon the presence of a root tip.

In a second experiment using both colchicine and indole-3-acetic acid (heteroauxin) the series of seedlings were treated as follows:

(1) $M/1000$ colchicine + 0·01 % indole-3-acetic acid together for 6 hr. after having previously removed the root tips.

(2) $M/1000$ colchicine for 6 hr., then 0·01 % indole-3-acetic acid for a further 6 hr. after having previously removed the root tips.

(3) No treatment; root tips removed.
(4) As (1) but root tips not removed.
(5) As (2) but root tips not removed.
(6) Control: no treatment.
(7) 0·01 % indole-3-acetic acid for 6 hr.; root tips removed.
(8) 0·01 % indole-3-acetic acid for 6 hr.; root tips not removed.
(9) $M/1000$ colchicine for 6 hr.; tips removed.
(10) $M/1000$ colchicine for 6 hr.; tips not removed.

No swellings were obtained in those samples where the root tips had been removed before treatment (i.e. numbers 1, 2, 3, 7 and 9), or, of course, in the controls. The typical colchicine swellings were seen in samples 4, 5, 8 and 10, that is, in those samples with unmutilated root tips treated with colchicine or heteroauxin or both. The effect was not so pronounced in sample 5. This experiment confirms the previous one that colchicine and heteroauxin only produce a swelling behind the root tip when that organ is still intact. It might be of interest, however, to see whether decapitated stumps would swell up under the influence of colchicine and heteroauxin when the tips were reapplied.

It is possible that the slowing down and cessation of cell division under the influence of colchicine causes a large excess of growth substances to accumulate which would normally be used up by the actively growing and expanding cells. Such a situation would also be arrived at by

treatment with excess quantities of heteroauxin. The action of this excess of growth substance seems to be the destruction of polarity and the extension of the cell walls in all directions. The precise mechanism of this action however is as yet quite obscure.

SUMMARY

Treatment of *Allium Cepa* seedlings by colchicine was found to be most effective for producing chromosome doubling in concentrations of $M/1000$ (0·04 %) to $M/100$ (0·4 %). During mitosis the spindle mechanism is inhibited but can recover its normal function after mild treatment. The only cytological effect of the drug is apparently on those stages where the spindle would normally be present. Contrary to Levan's statements the centromere of the *c*-pairs rarely divides until the resting stage has been entered. At the end of the *c*-metaphase the chromosomes are reformed into a lobed restitution nucleus very distinct in form from the ordinary resting nucleus. Hence the number of restitution nuclei in a root gives a direct indication of how many cells have divided whilst under the influence of the drug. Those cells which have passed through more than one division under colchicine influence are incapable of many further divisions, since the resulting plants never possess more than double the normal number of chromosomes.

The division cycle is greatly lengthened to about 33 hr., the longest phases being the blocked *c*-metaphase and early restitution phase. This time is about twice as long as normal divisions under similar conditions.

The subterminal root swellings are shown to be due not, as Levan supposed, to an increase in cell volume consequent on a greatly increased chromosome complement but to a lack of polarity in the diploid cells behind the root tip. Instead of growing longitudinally, the maturing cells do so in all directions, their volumes remaining roughly the same as those of the cells in untreated roots.

It is also shown that a similar effect of subterminal root swelling is produced by heteroauxin treatment and that neither colchicine nor heteroauxin can produce any subterminal swellings if the root tips are removed.

My thanks are due to Dr D. G. Catcheside for his constant help and advice during the course of the investigations.

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EXPLANATION OF PLATE 1

Fig. 1. *Allium Cepa*. Photomicrographs of the cell shown diagrammatically in Text-fig. 3 in three different planes of focus. The relic spirals can be quite plainly seen in several chromatids. ×1100.

Fig. 2. *Allium Cepa*. The macroscopic effect of colchicine. *a*, untreated seedlings; *b*, seedlings of the same age after 10 days' colchicine treatment. The scale is in centimetres in both *a* and *b*.

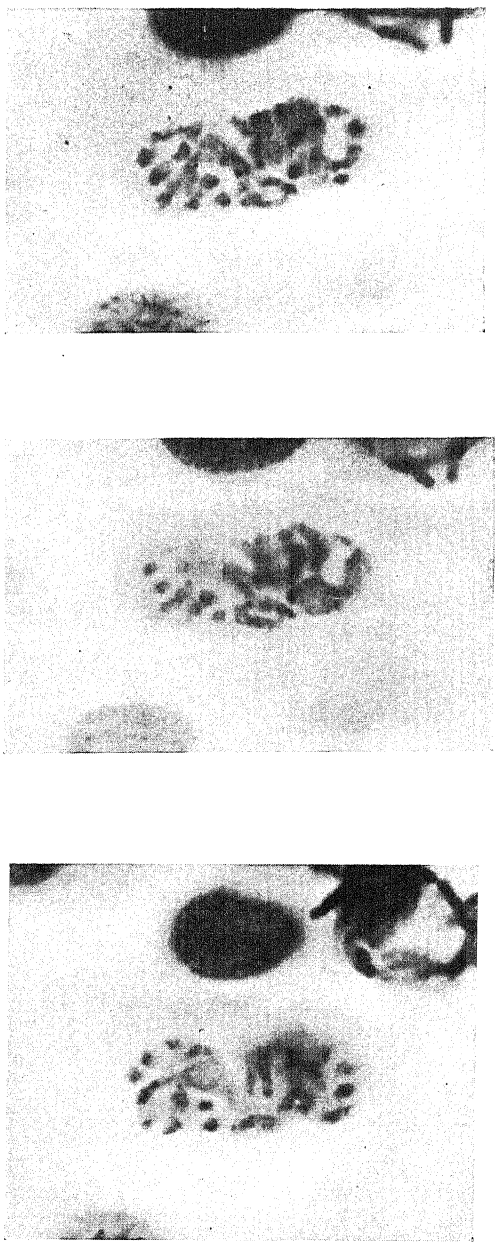
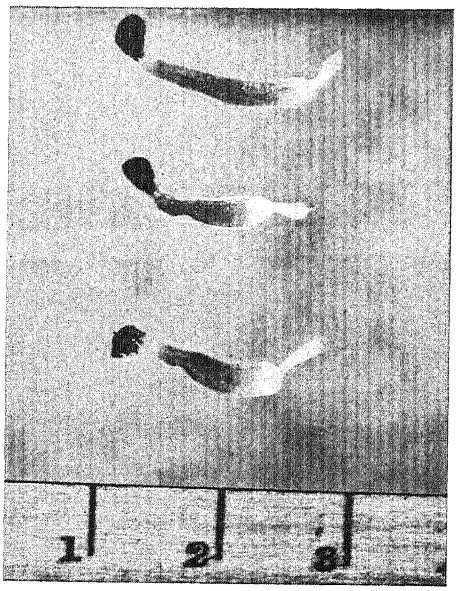
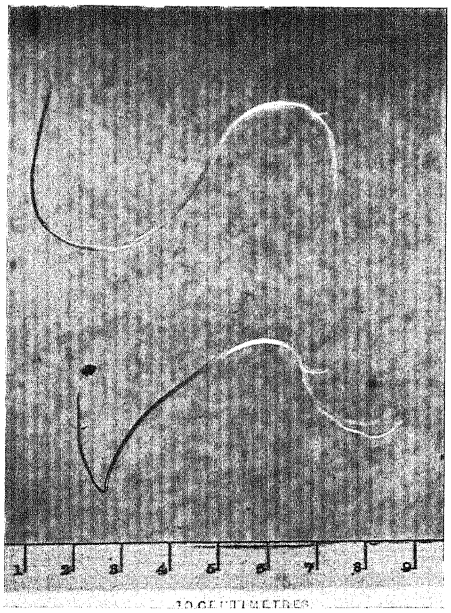


Fig. 1.



b



a

Fig. 2.

INTERGENERIC HYBRIDS OF *SACCHARUM*

IV. *SACCHARUM-NARENGA*

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(With Seven Text-figures)

CONTENTS

1. History of the cross

In Parts I–III of this series I have described what happens when high polyploid species of *Saccharum*—*S. officinarum* ($2n=8x=80$), *S. spontaneum* 'Glagah' ($2n=112$) or their derivatives, like POJ 2725 ($2n=106$) —are crossed with diploid species of *Erianthus* ($2n=20$), *Imperata* ($2n=20$) and *Zea* ($2n=20+2B$). I now come to an intergeneric hybrid of *Saccharum* in which the male parent, *Narenga porphyrocoma* Hance (Bor.), is a hexaploid. This cross was made under controlled conditions by the late C. A. Barber at Coimbatore in 1913. Its low sucrose content as compared with others of Barber's crosses made it worthless as a substitute cane, while its complete sterility prevented any further use of it as a parent. Its propagation as a possible economic cane was therefore stopped after a detailed recording of 100 F_1 seedlings had been made (Barber, 1916).

About a score of these seedlings were, however, grown at the Imperial Sugar Cane Station as 'an interesting demonstration and in the hope that at some time their fuller examination may be taken up', to quote Barber (1920). The present paper is the outcome of such a study, made on a few of these surviving hybrids and their parents.

2. Material and methods

Narenga porphyrocoma Hance (Bor.), until recently known as *Saccharum Narenga*, is a tall perennial grass found widely in north-east India. I collected many clones of it in 1937 from Assam, where I have seen it flowering profusely on the banks of the Brahmaputra. According to Barber (1916) the male parent he used for crossing with *S. officinarum* was raised from seeds collected in north Bihar. This plant was being propagated from cuttings at Coimbatore, so that I was able to examine the identical clone used by Barber in 1913. I have also examined clones I collected in Assam, as well as the herbarium sheets at Kew. Six of these clones proved to have 30 chromosomes.

The *S. officinarum* clone studied was the clone used by Barber in his cross. It was the same clone of Vellai which I used for crossing with *Zea Mays*.

Material for cytological studies was grown and collected at Coimbatore. The technique was the same as that described in previous papers. Permanent acetocarmine smears were used for meiotic studies.

3. General characters of parents and F_1 hybrids

According to Bor (1940) the retention of '*Saccharum Narenga*' in the genus *Saccharum* was anomalous, owing to its possessing morphological characters quite distinct from those species accepted as members of that genus. The glumes are more coriaceous, there are no non-flowering stems; the general appearance of the plant is flimsier; the inflorescence, which in *S. officinarum* is a large panicle, is very reduced in *Narenga* (Fig. 1), and only the lowest lateral axis bears secondary branches. There is a fourth glume which is absent in *Saccharum officinarum*.

All the hybrids between *Saccharum* and *Narenga* are very cane-like. Unlike the *Saccharum-Zea* cross they are extremely vigorous, and Barber (1916) reported that they flowered at 10 months from germination.

In quantitative characters such as diameter of stem, width of leaves, size and branching of inflorescence and length of callus hairs, the F_1 plants were mostly intermediate between the parents (Figs. 1, 2). But it would appear from Barber's analysis of 100 seedlings that they showed considerable variation among themselves. This is especially marked in his photograph of stems (1916), where he has a class of seedlings which equalled the *Saccharum* parent in stem diameter (Fig. 3). This type was not represented in the hybrids I examined cytologically. Barber's analysis also shows considerable variation in the percentage of sucrose

present in the 100 seedlings. The majority had about 11%, though some had as little as 6%.

In Table 1 I have summarized the general qualitative characters of taxonomic value noted in the parents and the hybrids. It will be seen

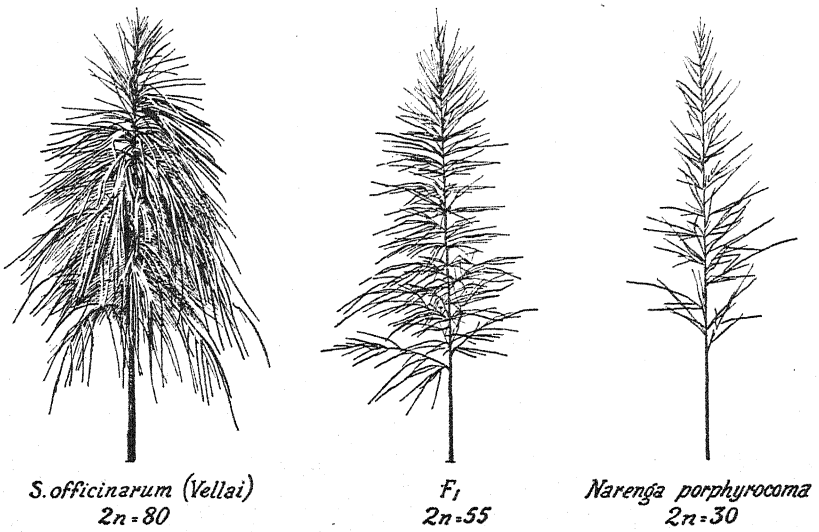

Fig. 1. Inflorescence of *Saccharum officinarum* (Vellai), *Narenga porphyrocoma* and F_1.

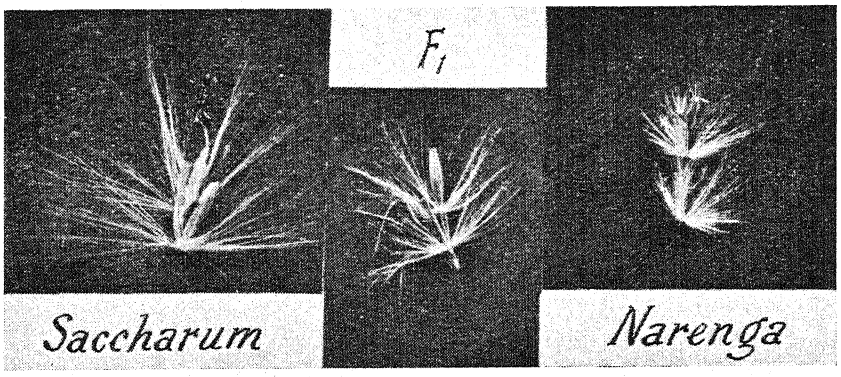

Fig. 2. Spikelets of *Saccharum*, *Narenga* and F_1.

that the hybrids resembled *Saccharum* in five and *Narenga* in five of the contrasting characters, one character, the shape of the nodal buds, being intermediate. One character is not intermediate: the minute cilia on the lodicules, lacking in *Saccharum* but present in *Narenga*, were very much exaggerated in all the F_1 hybrids. In the dominance of the epidermal hairs on the leaf and the ligular process (Fig. 4) the

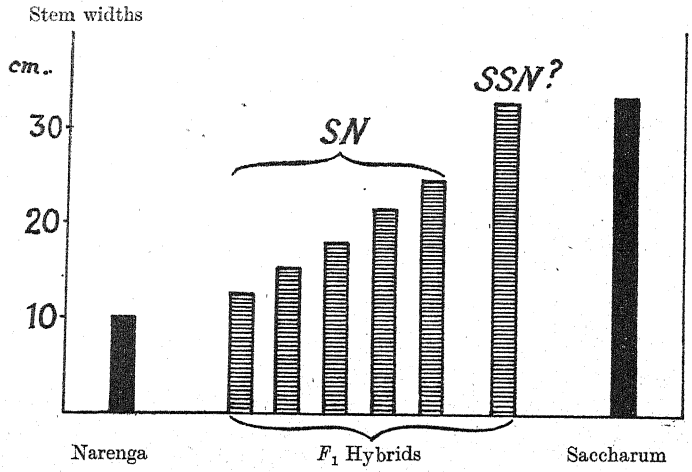

Fig. 3. Relative stem widths of *Narenga*, *Saccharum* and their F_1 hybrids (measurements after Barber).

Table 1. *Comparison of characters of* Saccharum officinarum (*Vellai*), Narenga porphyrocoma *and* F_1 *hybrids*

	Saccharum	F_1	*Narenga*
1. Habit	Perennial	Perennial	Perennial
2. Stem anatomy	Nodes and internodes present	←	Short rhizome Aerial stem develops during flowering only
3. Root eyes	Present	←	Absent
4. Bud	Ovate	Lanceolate	Elliptical
5. Ligular process	Present	←	Absent
6. Leaf blade	Non-fluted	←	Fluted
7. Upper epidermis	Non-hairy	→	Hairy
8. Main axis of inflorescence	Non-hairy	→	Hairy
9. Fourth glume	Absent	→	Present
10. Glumes	Membranous	→	Coriaceous
11. Callus hairs	Longer than glume	←	Equal to glume
12. Lodicules	Non-ciliate	→	Ciliate

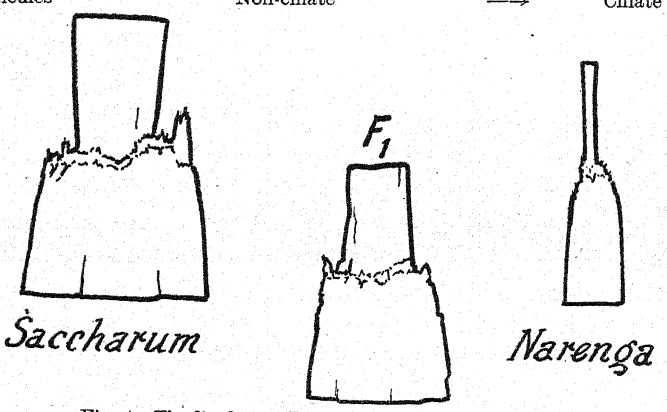

Fig. 4. The ligule in 'Vellai', *Narenga* and F_1 hybrid.

Saccharum-Narenga hybrids are similar to the *Zea* hybrid (Janaki-Ammal, 1941).

The generic character of coriaceous glumes in *Narenga* was modified to 'thinly coriaceous' in the hybrid.

4. CYTOLOGY OF PARENTS AND F_1 HYBRIDS

(*a*) *Somatic chromosomes*

The chromosome number of Vellai, $2n=80$ (Fig. 5*a*), has been recorded in the previous studies of this series. *Saccharum officinarum* is regarded as an octoploid. The 80 chromosomes of Vellai could be broadly classified into four types with regard to length. Secondary constrictions were found in the long chromosomes.

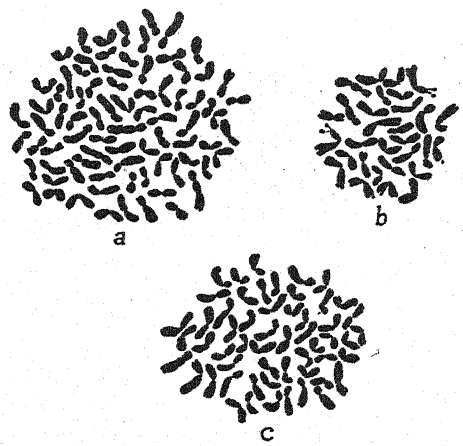

Fig. 5. Root-tip metaphase in (*a*) *Saccharum officinarum* (Vellai) ($2n=80$), (*b*) *Narenga porphyrocoma* ($2n=30$), (*c*) *Saccharum-Narenga* hybrid ($2n=55$). ×2000.

Root tips of all the clones of *Narenga porphyrocoma* examined had 30 chromosomes (Fig. 5*b*). This number verifies the count of Bremer (1925) from the 15 bivalents seen in pollen mother cells of this plant. It is the only genus in the Andropogoneae with this number. Its separation from *Saccharum* on morphological grounds by Bor (1940) can thus be supported cytologically.

Secondary constrictions were seen in the long chromosomes as in *Saccharum*. A single pair of chromosomes have trabants.

In root tips of sixteen of the hybrids I found 55 chromosomes (Fig. 5*c*). This number represents the sum of the haploid numbers of Vellai and *Narenga*. As the cane Vellai when crossed with *Sorghum Durra* produces both diploid and triploid hybrids ($2n=50$, 90; Janaki-Ammal, 1941), it

is to be presumed that only haploid egg cells of Vellai are fertilized by *Narenga*. In this respect the *Saccharum-Narenga* hybrids are similar to *Saccharum-Zea*. Such selective fertilization, or selective survival of fertilization types, seems to be characteristic of sugar-cane hybrids (Janaki-Ammal, 1941).

(*b*) *Meiosis and male sterility in* S. officinarum (*Vellai*)

Pollen mother cells of Vellai at diakinesis showed that the 80 chromosomes associate to form 40 bivalents (Fig. 6*a*). They form one or two chiasmata only. Occasionally two of the chromosomes are seen unpaired at metaphase. They then fail to congress on the metaphase plate, and

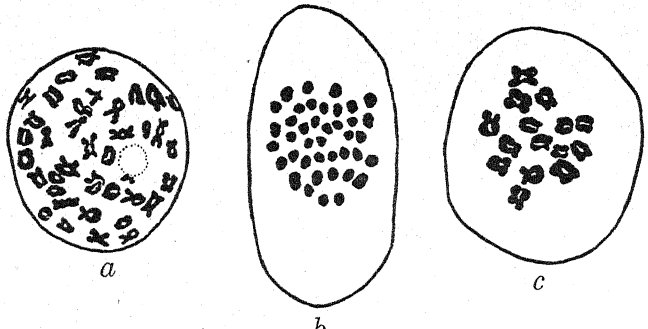

Fig. 6. (*a*) Pollen mother cells at diakinesis in Vellai. ×1800. (*b*) Polar view of metaphase II in pollen mother cells of Vellai. ×1800. (*c*) Prometaphase in pollen mother cells of *Narenga*. ×2000.

this may be responsible for the unequal numbers observed at metaphase II by Dutt & Subba Rao (1933). Except for this abnormality metaphase I is regular. As a rule 40 chromosomes were also counted at metaphase II (Fig. 6*b*). Tetrad formation is regular, though occasionally I came across triads in which one of the cells is binucleate. Anthers after tetrad formation showed a progressive deterioration in the pollen grains, generally beginning before the first division in the pollen grain. At this stage there is normally a change in the cytoplasm of the pollen grains, and starch grains are developed. In the degenerating pollen grains of Vellai starch is either poorly developed or totally absent. All such cells abort. About 99% of pollen grains in open spikelets were found to be aborted. As less than 2% of the anthers in an inflorescence burst, the pollen fertility is finally reduced to zero. That viability of the embryo-sac is not impaired is shown by the 200 seedlings obtained by Barber in this cross.

(*c*) *Meiosis in* Narenga porphyrocoma

The 30 chromosomes of *Narenga* form 15 bivalents (Fig. 6*c*), as recorded by Bremer (1925). Reduction division is regular, and pollen tetrads and grains are formed in the normal way. Pollen fertility is nearly 100%. On this evidence it is regarded as a hexaploid plant with a basic number of 5 instead of 10. Diploids of this basic number in the Andropogoneae are found only amongst the para-Sorghums.

(*d*) *Chromosome behaviour in* F_1 *hybrids*

Meiosis was studied in five seedlings. Pollen mother cells at diakinesis showed that the 55 chromosomes associate as bivalents, trivalents and quadrivalents (Fig. 7*a*). Table 2 gives the configurations noted in fifteen cells of three F_1 hybrids. My observations do not agree with those of

Table 2. *Degree of association in the three* F_1 *hybrids, A, B and C*

Configurations				No. of cells in			
IV	III	II	I	*A*	*B*	*C*	Total
2	2	19	3	5	1	1	7
2	1	20	4	5	5	3	13
2	0	21	5	2	1	4	7
1	2	21	3	3	2	0	5
1	1	22	4	0	4	2	6
1	0	24	3	0	0	2	2
1	0	23	5	0	2	3	5
							45 cells
Average 1·5	1	21	4				
% in *A* 6·7	5·3	74·6	13·4				
% in *B* 5·3	3·6	76·5	14·6				
% in *C* 5·5	1·6	77·7	15·2				

Singh (1934), who has recorded only bivalents and univalents in one plant he examined. The large number of bivalents present in the hybrid (19–23) shows that the chromosomes derived from the haploid complement of *Saccharum officinarum* are capable of pairing amongst themselves (by autosyndesis), like the *S. spontaneum* chromosomes in the cross with *Erianthus* (Janaki-Ammal, 1941). The percentage of configurations is fairly uniform for the three plants studied.

(*e*) *Behaviour of univalents*

The unpaired chromosomes in the hybrid, 3–5 in number, appear to be the largest of the complex. They probably belong to the *Narenga* parent. These chromosomes tend to be pushed towards the periphery of the nucleus even at the diakinesis stage. They are therefore at a positional disadvantage when the spindle is formed. They are always found on the edge of the spindle, where the forces of congression are apparently not so effective (Fig. 7*b*). The fate of the univalents during

meiosis depends on the degree of congression at metaphase I. Those univalents that are able to reach the plate in time divide at metaphase I (Fig. 7*c*) and generally lag at the second division. These lagging univalents move only through the agency of the stretching spindle, and not all of them reach the daughter nuclei of the tetrad stage.

Those univalents which are outside the effective sphere of action of the spindle at metaphase I either remain at the poles and become incorporated in the daughter nuclei, or may remain undivided at the plate.

In the first case they will divide normally at the second division, while in the second case they are seen to form a separate nucleus (Fig. 7*d*). Development is somewhat slower in these extra nuclei than in the main nucleus. They may form their own spindles at the second division (Fig. 7*e*), and then dyads with two micronuclei are seen (Fig.7*f*).

There are usually five chromosomes—the maximum number of univalents observed—in these extra nuclei, indicating that they are formed when the general congression is weak.

The behaviour of the haploid chromosomes in *Narenga* in the hybrid is thus similar to that in a triploid plant with 5^{II} and 5^{I}.

Pollen sterility is very high, over 90%, in the hybrids. The few viable pollen grains seen in the anthers are not available as the anthers do not dehisce. The hybrids are also female-sterile.

The close resemblance between Barber's hybrids and the wild cane Hitam Rokhan (hitam meaning red) indicates the same origin. This cane, described by Bremer in 1925, was collected by J. B. Haga in 1916 on the banks of the river Rokhan in eastern Sumatra. It has the same chromosome number, $2n=55$, as Barber's hybrids. It differs from them in the degree of autosyndesis of the chromosomes (Bremer, 1925). This is probably due to its being a hybrid of a different clone of *Saccharum officinarum*. The red colour of the cane points to one like Black Cheribon as the possible female parent.

Similarly the mosaic-resistant cane Kassoer, also found in the East Indies, is a natural hybrid between *S. officinarum* Black Cheribon and *S. spontaneum* (Bremer, 1923). Thus the two hybrids made by Barber in 1913 reproduce types occurring naturally. While the interspecific hybrids, both natural and artificial, proved of immense importance to the sugar-cane industry of Java and India, the intergeneric hybrids because of their sterility had to be discarded from all breeding programmes. It is to be hoped that the use of colchicine and other drugs for the production of amphidiploids will make even the sterile hybrids of some use.

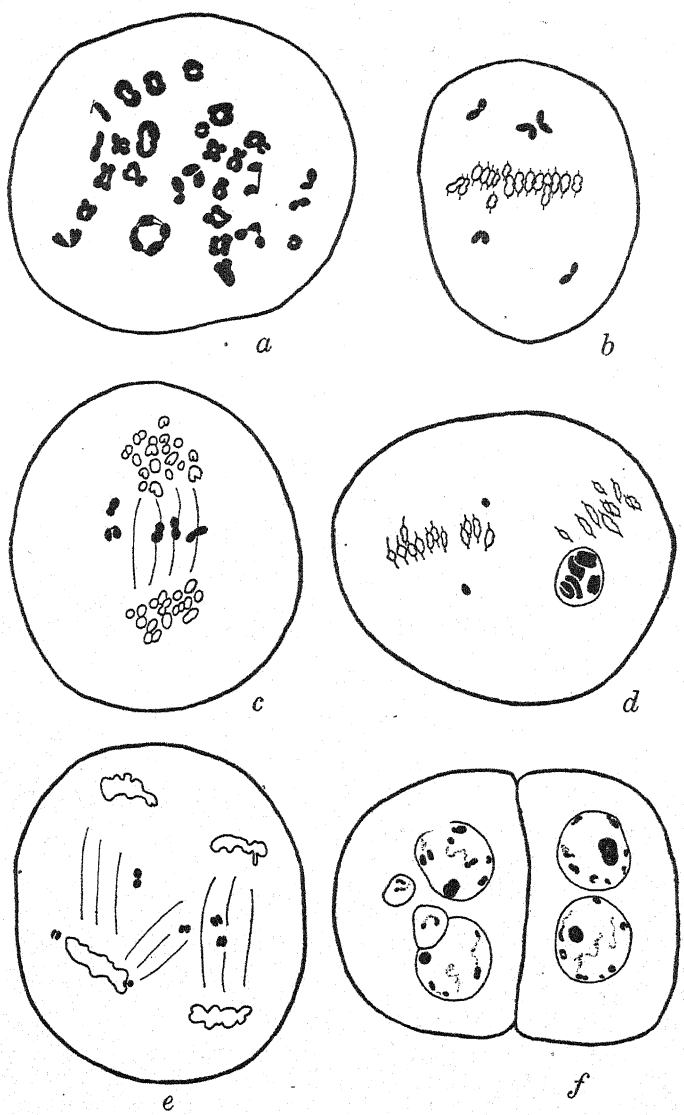

Fig. 7. Meiosis in *Saccharum-Narenga* hybrid. (*a*) Chromosome association at diakinesis in pollen mother cells. (*b*) Metaphase I showing position of univalents. (*c*) Telophase I with univalents at equator of plate. (*d*) Metaphase II with micronucleus of five univalents. (*e*) Telophase II with extra spindle formed by dividing micronucleus. (*f*) Dyad with two micronuclei. × 1800.

5. Summary

1. The hybrids made by C. A. Barber in 1913 between *Saccharum officinarum* (Vellai), $2n=80$, and *Narenga porphyrocoma*, $2n=30$, have 55 chromosomes.

2. They show detailed qualitative characters of each parent, but in general appearance are more like sugar canes.

3. In quantitative characters the hybrids are generally intermediate between the parents; only the minute cilia present on the lodicules of *Narenga* and absent in *Saccharum officinarum* were longer in the F_1 hybrids.

4. The 30 chromosomes of *Narenga porphyrocoma* form 15 bivalents and behave normally at meiosis.

5. The 80 chromosomes of *Saccharum officinarum* (Vellai) form 40 bivalents. Meiosis is generally regular. Male sterility in this sugar cane is due to defects in pollen-grain division.

6. The chromosomes in the *Saccharum-Narenga* hybrids show autosyndesis and associate as quadrivalents, trivalents and bivalents, while a few (3–5) remained as univalents.

7. Univalents, which are probably derived from *Narenga*, divided at metaphase I or II according to the degree of congression at metaphase I. Those outside the sphere of influence of the main spindle form extra nuclei which divide as separate units.

8. Male and female sterility in the hybrids is presumably due to autosyndesis of the chromosomes of both parents.

9. The wild cane Hitam Rokhan is evidently a natural hybrid between *Saccharum* and *Narenga*.

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AUTOTETRAPLOID INHERITANCE IN THE POTATO: SOME NEW EVIDENCE

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(With Plate 2 and One Text-figure)

CONTENTS

Introduction

The detailed attention which most of the staple economic crops have received at the hands of cytologists and geneticists has not been shared by the potato. The reasons for this state of affairs are not entirely clear. As a family, the Solanaceae have long been a classical example of a polyploid series, and accurate counts of the somatic and gametic chromosome numbers of the potato ($2n=48$) were available so early as 1926 and 1927 (Campin in Salaman, 1926; Stow, 1927). It therefore comes as rather a surprise to find that geneticists chose to ignore the implications of this cytological evidence. The assumption that the potato behaved genetically as a diploid survived, apparently unquestioned, until the publication of Lunden's comprehensive paper in 1937. This author has identified, among others, seven genes connected with colour inheritance, five of which show, without doubt, tetrasomic segregations, and Lunden concludes that the potato is an autotetraploid.

Two papers have recently appeared in which segregations for pollen sterility (Krantz, Becker & Fineman, 1939) and immunity to virus infection (Stevenson, Schultz & Clark, 1939) have been explained on a tetraploid basis. In both cases, however, the number of plants and progenies dealt with are small and additional data are needed to substantiate the hypotheses put forward.

The object of this paper is to present further data in support of the contention that the potato is an autotetraploid.

The technical difficulties of polyploid material are largely due to the presence of polymeric genes and the extent of 'blending inheritance' which follows in consequence. It therefore becomes less easy to discern the segregational behaviour of individual genes upon which the successful analysis of the genetical behaviour of any material depends. There seems, however, to be no reasonable doubt that the character dealt with below is controlled by the allelomorphs of a single gene. The character in question is a type of reaction, termed top necrosis, which follows infection of the potato by certain viruses. In addition, as I hope to show, the top necrosis reaction has a very definite economic value in that it provides a form of immunity to infection by these particular viruses under field conditions.

The work was begun by Dr Cockerham at this Station in 1935, and he concluded that the ability to react with top necrosis to virus infection behaved as a dominant character (Cockerham, 1937, 1939). I am greatly indebted to him for allowing me both to carry on this work and to include data from a number of progenies scored by him previous to 1938. The responsibility for the interpretation of these data, however, is entirely mine. Though further work is necessary for the amplification of data and the clarification of certain points, it is believed that the investigation has reached a stage at which results may profitably be presented. For helpful criticism of the manuscript and permission to reproduce Text-fig. 1 thanks are due to Dr K. Mather.

Notes on the character of top necrosis

As it was felt that descriptive notes of the phenotypes dealt with would, of themselves, form but an inadequate background to the investigation, the virological aspects of the problem have been dealt with in some detail in this first section.

Within the space of a decade the study of plant viruses has evolved from a mere appendage to mycology to a branch of biology of almost appalling complexity. During this time, about fourteen viruses of varying degrees of economic importance have been discovered to attack the potato. The effects of only four of these, namely, viruses *X*, *A*, *B* and *C*,* are the immediate concern of this paper.

* Virus nomenclature at the present time is in a chaotic state since no one system has received general recognition. In the present account it has seemed best to use those names by which the viruses are best known.

Of these four, viruses *X* and *A* are of the greatest economic significance. Separately or together they are responsible for much of the 'mosaic' and 'crinkle' diseases which lead to the 'degeneration' or 'running out' of stocks of European and American potato varieties. Virus *B* is widespread among American commercial varieties but very little is known of its effects or those of virus *C*. (For more detailed information on these viruses and references see Smith, 1937.)

Under natural conditions virus *A* is transmitted from one plant to another by aphids (Loughnane, 1933) and virus *X* by leaf contact (Loughnane & Murphy, 1938), but the mode of transmission of viruses *B* and *C* is not fully known. If, however, potato plants are artificially infected by grafting to them scions containing any one of these four viruses, one of three things may happen. Either (*a*) there is scarcely any observable change in the appearance of the plant; or (*b*) the foliage develops a mosaic of varying degrees of severity (Pl. 2, fig. 3); or (*c*) a fine network of necroses appears near the shoot apices which, spreading through the stem destroys the growing points and eventually the whole plant (Pl. 2, fig. 1). To this last reaction Quanjer (1931) gave the name top necrosis. He, and later Bawden (1932), found that the disintegration and death of the cells which accompanied necrosis first appeared in the phloem, spreading later to the surrounding tissues. If top necrotic plants form any tubers at all these are usually spotted and blotched with black, necrotic patches. In storage the necrosis spreads to the tuber buds, and by the following season the tubers are frequently reduced to a mass of cork. Partially necrotic tubers produce apparently normal sprouts which soon become smothered with necroses to which the young plants succumb. Unblemished tubers produce healthy plants with no signs of necrosis. In most cases, then, the total effect of top necrosis is the elimination of the infected plant and its vegetative progeny.

Using pure stocks or viruses *X*, *A*, *B* and *C*, Cockerham (1937, 1939 and unpubl.) has graft infected a large number of potato varieties. He has found, in common with earlier workers on viruses *X* and *A* (Bawden, 1936; Murphy & McKay, 1932), that the behaviour of individual varieties to each of these viruses is quite characteristic. For example, the variety Craigs Defiance reacts with top necrosis to all four; Epicure is top necrotic to *X*, *A* and *C*; Duke of York to *A* and *C* only; Kerr's pink to *A* and *B*; Di Vernon to none of the four, and so on (Cockerham, 1937, 1939 and unpubl.).

Now, if the results of such grafting tests are collated with the behaviour of the same varieties under field conditions two very striking

facts emerge. First, plants showing top necrosis are a great rarity in field crops. In fact, this 'disease' is typically a 'laboratory disease' produced under the artificial conditions of graft infection. Secondly, it appears that, in the field, a variety never carries any virus which produces top necrosis in it on graft infection. Thus, it is never possible to extract viruses *X*, *A*, *B* or *C* from field samples of Craigs Defiance, *X*, *A* or *C* from Epicure and so on; though Di Vernon may carry all four viruses, and most commercial stocks of Duke of York carry viruses *X* and *B*. These facts invite the conclusion that immunity from infection by *X*, *A*, *B* or *C* in the field is in some way connected with the manner in which a given variety reacts to these viruses under graft infection. The problem is to demonstrate the causal connexion between the two.

It is possible, with varying degrees of success, to transfer all four viruses from one plant to another by rubbing or needle inoculating the leaves with virus-infected sap. The course of events after inoculation depends on whether the plant is tolerant or intolerant (top necrotic) to the infector virus. Tolerant plants become permeated by the virus, and the symptoms they develop do not differ from those following graft infection by the same virus. But, in the case of intolerant plants, either large black lesions develop on the inoculated leaves (Pl. 2, fig. 2), or, more rarely, no local lesions develop and a full top necrosis appears after a short time. When local lesions develop it is never possible to extract the infector virus from other parts of the plant. In other words, the virus appears to have been localized within the lesions. After a large number of seedlings had been leaf inoculated with virus *X* by Cockerham and the writer, it was found that nearly all plants which developed local lesions later reacted with top necrosis when this same virus was introduced by grafting. Attempts by Smith (1931) and Bawden (1936) to transfer virus *B* to President resulted in the production of necrotic lesions on the inoculated leaves, and neither worker succeeded in extracting virus *B* from other parts of the plant. Actually President proves to be top necrotic to virus *B* (Bawden, 1936, and others). Other examples of this association of localization with 'recovery' from virus infection might be quoted at length.

It follows, then, that a plant which is killed by a virus introduced by grafting may be 'resistant' to the same virus when this is introduced by leaf inoculations. Occasionally this 'resistance' breaks down; a full top necrosis develops and the plant is killed. The top-necrotic reaction is, then, really an expression of extreme susceptibility, of which the local lesion phenomenon is merely another aspect.

The situation appears less paradoxical if the nature of the two methods of infection, grafting and leaf inoculation, are considered in terms of the relative amounts of virus introduced into the plant. In both cases the reaction of the plant to the pathogen is a very intense one. But, whilst there may be an unrestricted flow of virus particles through a graft union, the number of particles successfully penetrating an inoculated leaf must be very small and the supply strictly limited in comparison. The virus is thus effectively localized at its point of entry by the lesions which develop on inoculated leaves. The evidence presented earlier shows that in the absence of such localization top necrosis does follow from leaf inoculation.

The amounts of virus transferred from one plant to another in the field by aphids (virus *A*) or by leaf contact (virus *X*) must likewise be comparatively small. Hence the conclusion seems irresistible that localization is the mechanism responsible for the 'resistance' of intolerant plants to their lethal viruses under field conditions.

This conclusion is one of very great importance. The incidence of top necrosis under field conditions is so slight that, practically speaking, intolerance is equivalent to immunity. Even if full top necrosis does occasionally develop, its effect in eliminating the virus-infected plant is still a beneficial one on the crop as a whole. The reaction of a plant when graft infected with viruses *X*, *A*, *B* and *C* is thus very definitely an index of its behaviour towards these viruses, and hence of its usefulness, under field conditions.

Whilst some few varieties have, accidentally, been produced which are top necrotic to all four viruses it is clearly of economic importance to know how to produce them at will.

Two facts simplify the genetical approach to the problem. In the first place, it is evident that top necrosis and its absence may be treated as a pair of clear-cut alternative characters. This holds for each of the four viruses *X*, *A*, *B* and *C*. Secondly, both these alternative kinds of reaction are to be found among potato varieties. Hence a study of the genetics of top necrosis should present no insuperable difficulties.

In this introductory survey emphasis has been laid on the essentially similar behaviour of the potato towards each of the viruses *X*, *A*, *B* and *C*. Attention will, however, be confined in the remainder of the paper to virus *X* top necrosis. Detailed consideration of the other three viruses must be deferred until sufficient data have accumulated.

Material and methods

Male and female sterility and the non-blooming habit are widespread among commercial varieties of potato and impose severe limitations on any breeding programme. In fact, the number of varieties which can be used as pollen parents is surprisingly small. Thus, as a rule, it is only possible to make reciprocal crosses between types and not between the same pair of varieties. Castration and bagging of all plants to be used as female parents have been carried out as routine precautions by the writer, though in most cases they could both actually be dispensed with as the chances of male sterile varieties being cross-fertilized are almost negligible. Potato flowers are extremely sensitive to excessive heat and humidity and they readily drop when enclosed in bags. As a compromise, therefore, flowers castrated in the bud stage are enclosed in pergamine bags punched with holes sufficiently small to keep out the larger visiting insects.

Something of the methods of collecting data on the inheritance of the top-necrotic reaction will have been gathered from what has gone before. Each plant of a progeny must be tested by grafting on to it a scion containing the particular virus one is studying and the type of reaction which follows noted. In raising material for test, the practice has usually been to sow the seed of desirable crosses early in the year previous to testing. The seedlings are planted out in the field and the tubers of each plant harvested and bagged separately in the autumn of that year. One tuber from each plant is potted up in the following year, beginning in February, and grown in an insect-proof greenhouse. The percentage of losses occurring in field-grown material is often inconveniently high. This technique has been considerably improved by raising seedlings and testing them in their first year, the whole process being carried out in the greenhouse. When, in either case, the plant reaches a convenient size for handling, one shoot is grafted with a scion of a variety known to carry the virus. All the progenies recorded here have been tested with scions from the same stock of Arran Victory containing virus *X*. Differences in virulence of the virus strain do not affect the expression of the top-necrotic reaction (Bawden, 1936).

Symptoms appear about 14 days after grafting. As to the actual details of scoring, there is no possibility of overlooking a plant with top necrosis, but the alternative reaction, a mosaic or mottle, does present difficulties. Union between stock and scion may not always be complete, and one has to make sure that the virus has actually passed from the

scion to the stock. Where an obvious mottle develops there is no doubt about the matter. In cases of uncertainty a scion from the doubtful plant is grafted to a test variety, one which is known to react with top necrosis if that particular virus is present. With persistently unsatisfactory results fresh tubers are set up and the plants retested. In this way the scoring can be made very accurate though the collection of data is slow and laborious. On the whole it is believed that there are very few doubtful or wrongly classified plants.

EXPERIMENTAL RESULTS

(*a*) *The nature of tetraploidy*

The generally accepted basic chromosome number of the Solanaceae is 12, so that the cultivated potato, with $2n=48$ chromosomes, is presumably a tetraploid. On the basis of the pairing behaviour of their chromosomes at meiosis two kinds of tetraploids are, very broadly, distinguishable, auto- and allotetraploids. As is familiar enough, these two kinds of polyploid differ fundamentally in their genetical behaviour. Consequently, some definite conclusion must be reached as to the precise nature of tetraploidy in the potato before the available genetical data can be adequately analysed. In order to arrive at such a conclusion it is therefore essential to know what genetical tests enable a rigorous distinction to be made between the typically disomic inheritance of an allotetraploid and the tetrasomic behaviour of an autotetraploid.

Data from phenotypic segregations only partially satisfy this criterion. With respect to a single pair of alleles, **Aa**, simplex (**Aaaa**) autotetraploids and simplex ($\mathbf{A_1a_1a_2a_2}$) allotetraploids, when backcrossed and selfed both give ratios of 1 : 1 and 3 : 1 dominants to recessives respectively.

On backcrossing and selfing, duplex (**AAaa**) autotetraploids give 5 : 1 and 35 : 1 ratios respectively. On the other hand, for duplex allotetraploids of the type $\mathbf{A_1a_1A_2a_2}$, where $\mathbf{A_1a_1}$ and $\mathbf{A_2a_2}$ are similar pairs of alleles but where strict autosyndesis prevails, the corresponding ratios are 3 : 1 and 15 : 1. These characteristic duplex segregations would appear, at first sight, effectively to distinguish tetrasomic from disomic inheritance. But, under certain conditions, later to be described, this distinction breaks down and autotetraploids may closely simulate the 3 : 1 and 15 : 1 segregations of an allotetraploid.

With this reservation in mind, the data assembled in Tables 1 and 2 and summarized in Table 3 may be examined. Table 1 shows the behaviour of crosses between necrotic and non-necrotic reactors. These

families fall into two groups according as the female or male parent is the necrotic reactor. Both groups are further subdivided on the basis of parentage. With the exception of the two Cardinal progenies, 51/*c* and 241/*a*, all the backcross families in Table 1 agree in showing about

Table 1

Genotype*	Family	Parentage	Nx	nx	Total
$Nxnx_3 \times nx_4$	50/*d*, *b*	Epicure × Shamrock	41	51	92
	224/*a*	Epicure × Alness	33	35	68
	260	Epicure × Argyll Favourite	14	22	36
	514/*a*, *b*	Epicure × Alannah	41	52	93
			129	160	289
	448/*b*	Epicure × Pepo	44	32	76
	48/*c*, *d*	Epicure × Pepo	10	12	22
	342/*a*	White City × Pepo	51	54	105
	413/*a*	Liddesdale Lads × Pepo	29	29	58
	415	Maud Meg × Pepo	72	84	156
	416/*a*	Southesk × Pepo	63	68	131
			269	279	548
	348/*a*, *b*	Benest × Alness	30	41	71
	457/*c*	Craigs Defiance × 70 (13)	36	51	87
	758/*b*	Craigs Defiance × Flourball	154	136	290
			220	228	448
$nx_4 \times Nxnx_3$	502/*a*	Di Vernon × Edgecote Purple	50	51	101
	506/*b*	Di Vernon × Liddesdale Lads	44	61	105
	520/*b*	Majestic × L 55	50	63	113
			144	175	319
$nx_4 \times Nx_2nx_2$	51/*c*	Golden Wonder × Cardinal	74	12	86
	241/*a*	Abundance × Cardinal	70	26	96
			144	38	182

* In conformity with genetical convention the ♀ parent is placed first.

Table 2

Genotype	Family	Parentage	Nx	nx	Total
$Nxnx_3 \times Nxnx_3$	267	Edgecote Purple, B.S.*	15	8	23
	129	Liddesdale Lads, N.S.†	107	33	140
			122	41	163
$Nx_2nx_2 \times Nx_2nx_2$	26/*c*, *d*	Cardinal, B.S.	44	3	47
	261/*a*	Cardinal, B.S.	24	5	29
	272/*a*	Cardinal, B.S.	27	2	29
	418/*a*	Cardinal, B.S.	27	0	27
			122	10	132

* B.S. = bagged self. † N.S. = natural self.

equal numbers of necrotic and non-necrotic plants. Table 3 shows that for this group of data there is no heterogeneity either between crosses or between reciprocals. As regards the two exceptional families 51/*c* and 241/*a*: separately they show 5 : 1 and 3 : 1 ratios respectively, jointly their segregation approaches a 3 : 1. This difference in behaviour between Cardinal and the other necrotic parents is confirmed by the segregations

in Table 2. The Cardinal selfed families agree relatively well with a 15 : 1, the Edgecote Purple and Liddesdale Lads families with a 3 : 1 ratio of necrotics to non-necrotics. These facts are accounted for if Cardinal is duplex for the dominant allele of a gene **nx** for which the remaining necrotic varieties are simplex. Since duplex and simplex genotypes are distinguishable only by their breeding behaviour, one dose of the **Nx** allele is sufficient for complete dominance. However, it will at once be seen that the duplex segregations do not provide critical evidence on the nature of tetraploidy.

The information required rigorously to distinguish disomic from tetrasomic inheritance is supplied only by the following tests. Selfing a simplex genotype gives, in all three cases, diploid, **Aa**, allotetraploid, $\mathbf{A_1a_1a_2a_2}$, and autotetraploid, **Aaaa**, a 3 : 1 ratio of dominants to recessives, or nearly so. But in the disomic case, diploid and allotetraploid, one-third of the individuals in the dominant class will be homozygous

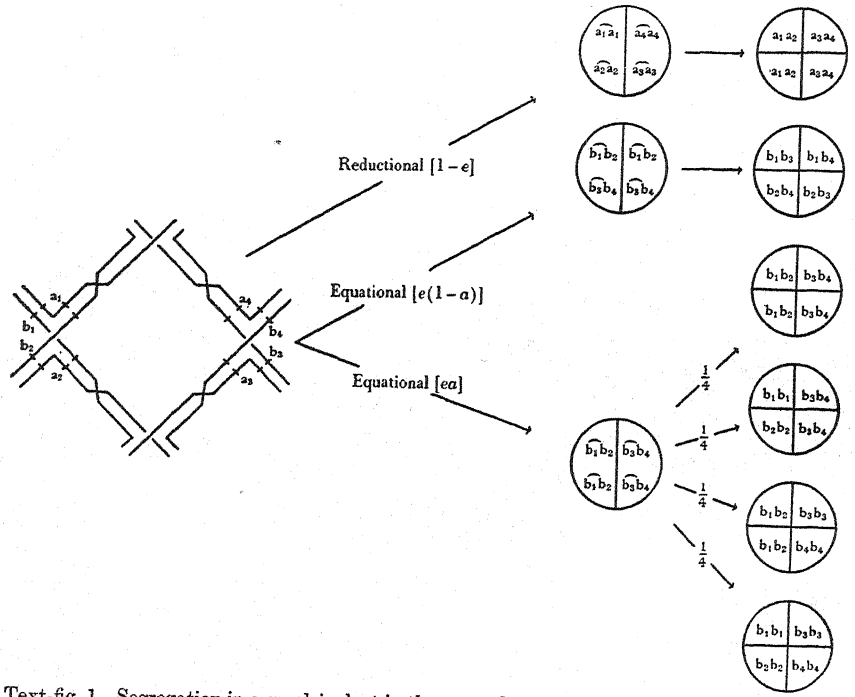
Table 3

	Expected		Observed		Deviation			Heterogeneity: Between crosses			Heterogeneity: Between reciprocals		
Genotype	**Nx**	**nx**	**Nx**	**nx**	χ^2	D.F.	P	χ^2	D.F.	P	χ^2	D.F.	P
$\mathbf{Nxnx_3 \times nx_4}$	1	1	618	667	1·8685	1	0·2 –0·1	11·0400	12	0·7 –0·5	0·8910	1	0·5–0·3
$\mathbf{nx_4 \times Nxnx_3}$	1	1	144	175	3·0125	1	0·1 –0·05	1·2453	2	0·7 –0·5			
$\mathbf{Nxnx_3 \times Nxnx_3}$	3	1	122	41	0·0020	1	0·98–0·95	1·3243	1	0·3 –0·2			
$\mathbf{nx_4 \times Nx_2nx_2}$	3	1	144	38	1·6484	1	0·2 –0·1	4·1207	1	0·05–0·02			
$\mathbf{Nx_2nx_2 \times Nx_2nx_2}$	15	1	122	10	0·4748	1	0·5 –0·3	7·4321	3	0·1 –0·05			

in the sense that they will not segregate again. In the tetrasomic case such non-segregators will be very rare or absent. An extension of this test serves to distinguish the two types of inheritance in the duplex phase. Selfing a duplex allotetraploid, $\mathbf{A_1a_1A_2a_2}$, will give a progeny in which approximately half the dominant class will consist of non-segregators. In the progeny of a selfed duplex autotetraploid the proportion of such dominant types will be one-fourth if triplex, **AAAa**, individuals are included. However, triplex types may betray their constitution through the occasional segregation of recessives (Blakeslee, Belling & Farnham, 1923; Lawrence, 1931; Lawrence & Scott-Moncrieff, 1935; de Winton & Haldane, 1935), in which case quadruplex plants will be the only non-segregators. These will not occur more frequently than once in thirty-five cases. Data from these tests are not yet available for the present material.

Under certain conditions, however, tetrasomic inheritance is distinguishable by a third means. The segregations of individual genes in autotetraploids frequently show characteristic deviations from the simple

autotetraploid expectations. From the nature of these deviations it is possible to deduce the pairing behaviour at meiosis, and hence the extent of homology, of the chromosomes in which such genes are located. The basis for such an inference is easily explained with the aid of Text-fig. 1.



Text-fig. 1. Segregation in a quadrivalent in the general case. Two loci are shown as marked each with four different allelomorphs. The two linked allelomorphs in the interphase nuclei are the two which are joined to the same attachment at first division. They must then separate at second division. Locus **a** shows reductional separation at the first division (top) and so cannot give double reduction, *i.e.* two identical allelomorphs in the same gamete. Locus **b** shows equational separation as a result of crossing-over between it and the spindle attachment. In some of the cases (middle) the equationally separating chromosomes reach different interphase nuclei and so double reduction is again impossible. In other cases (bottom), however, the two equationally separating chromosomes reach the same interphase nucleus, and double reduction occurs in half the cases for any allelomorph. The symbols e and a are the mean frequencies of equational separation and non-disjunction at the first division respectively (see in text), and the figure shows that double reduction occurs in only ae of cases. (From Mather, 1936.)

The locus **b** is represented as always having one chiasma between it and the centromere. After crossing-over has occurred, the two daughters of one allele will be attached to different centromeres. Chromosomes which have crossed over may then pass to the same or to opposite poles at anaphase. In the latter event the three possible types of interphase nuclei, $(\widehat{b_1b_2}\widehat{b_3b_4} : \widehat{b_1b_2}\widehat{b_3b_4})$, $(\widehat{b_1b_3}\widehat{b_2b_4} : \widehat{b_1b_3}\widehat{b_2b_4})$ and $(\widehat{b_1b_4}\widehat{b_2b_3} : \widehat{b_1b_4}\widehat{b_2b_3})$,

occur with equal frequency and will give the gametes, ($\mathbf{b_1b_2}$), ($\mathbf{b_1b_3}$), ($\mathbf{b_1b_4}$), ($\mathbf{b_2b_3}$), ($\mathbf{b_2b_4}$) and ($\mathbf{b_3b_4}$), in equal numbers. Where the two chromosomes pass to the same pole an important new possibility is introduced.

Since the two daughters of one allele are now attached to different centromeres they may behave independently of one another. In half the cases, therefore, they will both enter the same gamete. With four different alleles and the behaviour of the two interphase nuclei uncorrelated there are four equally probable types of behaviour at second anaphase (Text-fig. 1). This sequence of events leading to the production of gametes homozygous for a given allele was predicted by Darlington (1929) and termed by him 'double reduction'. Its occurrence will be marked in duplex and simplex backcrosses, where, as in the present case, one allele is dominant over the other by an excess of recessives over the expected 5 : 1 and 1 : 1 ratios respectively. In addition, duplex individuals will appear in simplex backcross progenies, where, in the absence of double reduction, none would be expected. Thus, where double reduction occurs, duplex autotetraploid segregations will tend to resemble the corresponding segregations of a duplex allotetraploid.

If, then, crossing-over takes place between the locus of **Nx** and the centromere, proof of double reduction at this locus will equally be proof that pachytene pairing and crossing-over occurs at random between the four chromosomes concerned. They must therefore be extensively if not completely homologous.

A glance at the segregations in Table 1 reveals that, with two exceptions which may be ascribed to sampling variance, both simplex and duplex backcrosses consistently show an excess of recessives over the expected 1 : 1 and 5 : 1 ratios respectively. As would be expected, if this deviation is the result of double reduction, the conformity of the simplex reciprocal crosses to this general scheme indicates that the behaviour on male and female sides of the plant is alike. The data are extensive enough to make the correctness of this explanation tolerably certain.

Absolute proof of the occurrence of double reduction is afforded by the detection of exceptional duplex plants in the backcross progeny of a simplex genotype. Authoritative evidence on this point is lacking in the present case, since complete classification of the dominant phenotypes of a backcross progeny is only possible once their breeding behaviour is known. The parentage of the duplex variety Cardinal might throw useful light on the situation but is, unfortunately, quite unknown.

On the other hand, the aberrant backcross ratios have no simple explanation on any allotetraploid hypothesis of pairing between the four **nx** carrying chromosomes. They do strongly suggest that these chromosomes are extensively homologous and pair at random, and consequently that, in analysing data on the segregation of genes located in these chromosomes, the potato may be regarded as an autotetraploid. The argument for autotetraploidy must inevitably be impaired by the lack of critical evidence which would irrefutably decide the issue. This lack is acutely felt and will necessarily take some little time to remedy. Nevertheless, the hypothesis does supply a completely satisfactory explanation for an otherwise obscure situation.

An additional check on the validity of this conclusion would be provided by quantitative data on the frequency of quadrivalent formation at meiosis, since double reduction will, as a rule, only follow from the association of all four homologues. The published data are, however, highly unsatisfactory. In the only two reliable accounts, those of Meurman & Rancken (1932) and Ellison (1936), quadrivalents and higher multivalents are described without any information as to their frequencies of occurrence. This point has been taken up by the writer. Numerical data are not yet available, but the frequency of quadrivalents is sufficiently high to suggest that the autotetraploid conclusion is well founded.

(*b*) *Analysis of data*

These preliminary considerations show that the genetical behaviour of autotetraploids will be more adequately described if some means of estimating the extent of double reduction at individual loci can be found. This question has been considered very fully by Mather (1935*a*, 1936) in two papers to which the reader is referred. His arguments are briefly these.

The two conditions for double reduction are (*a*) equational separation at the locus concerned, i.e. at first anaphase the two daughters of one allele must be attached to different centromeres, and (*b*) genetical non-disjunction of chromosomes which have crossed over at pachytene. 'The amount of double reduction will thus be a function of the product of the frequencies of occurrence of these phenomena' (Mather, 1936).

If the mean frequency of equational separation for a given locus be **e** and the frequency with which equationally separating chromosomes pass to the same pole at first anaphase be **a** then both conditions for double reduction will be satisfied in **ae** of cases.

In the case of a plant simplex for a given pair of alleles it can be shown that, where different grades of dominance are indistinguishable phenotypically, the gametic output of such a plant will be $(4-\mathbf{ae})$ dominants and $(4+\mathbf{ae})$ recessives. If **a** and **e** have the same values in male and female gametogenesis, the proportions of dominants and recessives on selfing will be $(48-8\alpha-\alpha^2)$ and $(16+8\alpha+\alpha^2)$ respectively, where α represents the product **ae** and is termed the simplex index of separation.

Applying this analysis to the present case a value for α may best be estimated by combining all the available data from simplex plants. As the methods of calculation have been adequately described elsewhere (Mather, 1935*b*, 1938), detailed repetition here would be superfluous.

The observed segregations are:

		Nx	nx	Total
$\mathbf{Nxnx_3} \times \mathbf{nx_4}$	Backcross	762	842	1604
$\mathbf{Nxnx_3} \times \mathbf{Nxnx_3}$	F_2	122	41	163

The value of α calculated from these data is 0·1770 and has a standard error of 0·0935. The agreement of the backcross and F_2 data with this joint estimate of α may now be tested. The heterogeneity χ^2 with one degree of freedom is 0·4163 with a probability of 0·7–0·5. The combination of the two sets of data is therefore quite legitimate.

Since there is, in a simplex plant, only one dominant-carrying chromosome, this must at any point be paired with an unlike recessive-carrying mate. In a duplex plant, however, there are two dominant-carrying chromosomes to be considered. Provided there are no changes of partner between the locus concerned and the centromere, in one-third of cases like chromosomes and in two-thirds of cases unlike chromosomes will be paired in this region. Clearly, double reduction can only occur in the two-thirds of cases where like and unlike chromosomes are paired. In this event the amount of equational separation, **e**, for either pair will be the same as in the simplex case; **a** will be the same if crossing-over in the two pairs is uncorrelated. The gametic segregation will then be $5-\mathbf{ae}$ dominants and $1+\mathbf{ae}$ recessives. If β represents the duplex index of separation these proportions on selfing become $(35-2\beta-\beta^2)$ dominants to $(1+2\beta+\beta^2)$ recessives. Using precisely the same methods as before a value for β may be calculated by combining all the available duplex data.

The observed segregations are:

		Nx	nx	Total
$\mathbf{nx_4} \times \mathbf{Nx_2nx_2}$	Backcross	144	38	182
$\mathbf{Nx_2nx_2} \times \mathbf{Nx_2nx_2}$	F_2	122	10	132

Whence $\beta = 0{\cdot}5071 \pm 0{\cdot}1533$.

The agreement of backcross and F_2 data with this estimate of β is not very good. The heterogeneity χ^2 for one degree of freedom is 3·1939 and has a probability of 0·1–0·05.

β is thus considerably but not significantly larger than α, as the following t test shows. t is calculated from the formula

$$t=\frac{\beta-\alpha}{\sqrt{(V_\alpha+V_\beta)}}$$

for $n_\alpha+n_\beta$ degrees of freedom, where n_α=number of plants in simplex progenies minus one, and n_β=number of plants in duplex progenies minus one, i.e. 2079 in this case, so that t may be treated as a normal deviate. $t=1{\cdot}838$, and the corresponding value of P, the probability, is 0·06–0·07, so the difference, though suggestively large, may be judged non-significant.

This difference in value between α and β is not entirely unexpected. The reason is to be sought in the effect of partner exchange on the proportion of equational separation at the **nx** locus.

Consider first the simplex case; with two chiasmata both involving the dominant-carrying chromosome and with no change of partner between them, there will be 50% equational separation at a locus beyond the second chiasma. If there is such a change of partner after the first chiasma, there will now be 100% equational separation, an increase of, at most, 50% (Mather, 1935*a*). In duplex plants a new situation is presented in the one-third of cases where both dominant-carrying chromosomes are paired. With two chiasmata and no change of partner between them there can clearly be no recognizable equational separation. Now, a change of partner in this case must always leave unlike chromosomes paired. Hence, after the change of partner, with one chiasma on each side of the exchange, a locus situated beyond the second chiasma will now show 100% equational separation. This 'should result in the duplex showing a greater increase in the number of recessive gametes over 1 in 6 than it does over 4 in 8 in the simplex. The excesses would be alike if there is no partner exchange' (Mather, 1936).

These predictions are fully borne out by the present data. Consequently the difference in value between α and β, though not statistically significant, is genetical evidence of the occurrence of partner exchange between the locus of **nx** and the centromere. This, in turn, indicates that the **nx** locus is situated some distance from the centromere. It is important to note that this is also cogent evidence against any hypothesis of preferential pairing among the four chromosomes concerned.

Finally, a word may be added on the general relations of α and β. When small they are expected to approximate closely, since small values indicate proximity of the gene locus to the centromere. The larger the values of the individual indices the greater should be the difference between them, since they will then indicate genes situated farther from the centromere (Mather, 1936). The series of α and β values in Table 4 agree quite well with these expectations. The indices of separation for two more unlinked genes in the potato have been calculated from Lunden's (1937) data. Evidently both of these are situated nearer to their respective centromeres than is **nx**.

Table 4

Gene pair	α	β
P, p	$0{\cdot}0565 \pm 0{\cdot}0812$	$0{\cdot}0691 \pm 0{\cdot}0316$
D, d	$0{\cdot}2228 \pm 0{\cdot}0105$	$0{\cdot}3076 \pm 0{\cdot}0603$
Nx, nx	$0{\cdot}1770 \pm 0{\cdot}0935$	$0{\cdot}5071 \pm 0{\cdot}1533$

There remains one more set of data to be discussed. The families set out in Table 5 have been dealt with separately on account of their relationship to the variety Kepplestone Kidney.

Selfed and used as a female parent this variety behaves as though simplex for the **Nx** allele. As a male parent, however, its behaviour is

Table 5

Genotype	Family	Parentage	**Nx**	**nx**	Total
$\mathbf{nx}_4 \times \mathbf{Nxnx}_3$	66	President × Kepplestone Kidney	50	50	100
	199	Kerr's Pink × Kepplestone Kidney	73	53	126
	200	Scot × Kepplestone Kidney	92	47	139
	201	Up to Date × Kepplestone Kidney	78	22	100
	205/*a*, *b*	Claymore × Kepplestone Kidney	93	64	157
	206/*a*	Peachbloom × Kepplestone Kidney	58	62	120
	263/*a*, *b*	British Queen × Kepplestone Kidney	45	27	72
	400	Arran Victory × Kepplestone Kidney	85	66	151
	404	Golden Wonder × Kepplestone Kidney	98	73	171
	405	Pepo × Kepplestone Kidney	41	25	66
	463	Arran Cairn × Kepplestone Kidney	91	74	165
			804	563	1367
$\mathbf{Nxnx}_3 \times \mathbf{nx}_4$	222	Kepplestone Kidney × Alness	7	13	20
	257	Kepplestone Kidney × 70 (13)	12	12	24
			19	25	44
$\mathbf{Nxnx}_3 \times \mathbf{Nxnx}_3$	71/*a*	Kepplestone Kidney, N.S.*	27	2	29
	109	Kepplestone Kidney, N.S.	52	21	73
	208	Kepplestone Kidney, B.S.†	13	6	19
	274	Kepplestone Kidney, B.S.	27	7	34
			119	36	155

* N.S. = natural self. † B.S. = bagged self.

definitely abnormal. Backcrosses of this type deviate significantly from the expected 1 : 1 ratio. With the exception of families 66 and 206/*a* the

deficiency is here on the recessive side, so that there can be no question of misclassification in this instance. We have clearly to deal with a case of anisogeny, the **nx** pollen grains being handicapped whilst the **Nx** pollen grains are not.

Two further points appear when these backcross segregations are analysed by the χ^2 method (Table 6). First, they do not form a homogeneous body of data, the heterogeneity χ^2 for nine degrees of freedom being 28·49. Secondly, the largest contributions to this heterogeneity χ^2 are made by families 66, 201 and 206/*a*. When these are removed the remaining families agree well in showing ratios of approximately 3 dominants to 2 recessives.

Table 6

Genotype	Expected	Observed		Deviation			Heterogeneity		
		Nx	**nx**	χ^2	D.F.	*P*	χ^2	D.F.	*P*
Kepplestone Kidney × $\mathbf{nx}_4$	1 : 1	19	25	0·8182	1	0·5–0·3	0·0182	1	0·9–0·8
$\mathbf{nx}_4$ × Kepplestone Kidney	1 : 1	804	563	42·4879	1	Very small	29·1882	10	<0·01
Kepplestone Kidney selfed	3 : 1	119	36	0·2602	1	0·7–0·5	6·1528	3	0·2–0·1

Table 7

Genotype		**Nx**	**nx**	Heterogeneity: Between groups χ^2	D.F.	*P*	Heterogeneity: Within groups χ^2	D.F.	*P*
$\mathbf{nx}_4$ × Kepplestone Kidney	66, 201 206/*a*	186	134	0·0821	1	0·8–0·7	23·6682	3	<0·01
	Remainder	618	429				5·3319	7	0·7–0·5

Such a behaviour would be expected if this variety is duplex **Nx** and pentasomic $\mathbf{Nx_2nx_3}$, i.e. it has 49 chromosomes, and that in competition with normal pollen $2n+1$ pollen is non-functional. Unequal segregation of quadrivalents is known to occur in the potato as in other tetraploids (Meurman & Rancken, 1932; Ellison, 1936). If the same assumptions are made about the relative viability of $2n$ and $2n+1$ ovules, the Kepplestone Kidney selfed families agree fairly well with expectations, but the two backcrosses with this variety as the female parent do not.

On the other hand, there is no heterogeneity between either of these latter two sets of families and the corresponding simplex selfs and back-crosses already dealt with (Table 8). Although the validity of the pentasomic hypothesis may be easily checked, more information on the

behaviour of Kepplestone Kidney as a female parent is needed before any useful conclusions can be drawn in any direction.

On account of these complications the Kepplestone Kidney data may not be used for an estimation of an index of separation.

Table 8

Genotype	Expected	Observed		Heterogeneity		
		Nx	**nx**	χ^2	D.F.	P
Kepplestone Kidney × $\mathbf{nx}_4$	1 : 1	19	25	0·3212	1	0·7–0·5
Others × $\mathbf{nx}_4$	1 : 1	762	842			
Kepplestone Kidney selfed	3 : 1	119	36	0·1574	1	0·7–0·5
Others selfed	3 : 1	122	41			

DISCUSSION

The principal conclusion which emerges from the genetical data is that the potato is autotetraploid for the chromosome carrying the **nx** locus. Evidence from double reduction of pairing and crossing-over between all four homologues at random though strong is admittedly incomplete. On the other hand, the fact that the simplex and duplex indices of separation do differ appreciably in magnitude is of the utmost significance. There seems no good reason to doubt the validity of the explanation already advanced for this fact. Hence, there is genetical evidence of partner exchange between the **nx** locus and the centromere, and the four chromosomes must therefore be extensively if not completely homologous.

A consideration of these findings, in conjunction with Lunden's (1937) data, is quite valuable. Lunden has identified seven pairs of genes controlling the inheritance of plant colour, all of them apparently unlinked. Confirmation of the correctness of his interpretations is very desirable. Nevertheless, five of these pairs, namely **Pp**, **Dd**, **Rr**, **Ff** and **Ss**, show typical tetrasomic segregations. Qualitative evidence of double reduction at the **d** locus is furnished by the recovery of nulliplex offspring from triplex, $\mathbf{Dd}_3$ individuals. Double reduction also occurs at the **p** locus. Failure to detect linkage between two genes is not of course proof of their location in different chromosomes. Hence, it would be unjustifiable to conclude that these facts demonstrate the extensive homology of more than one set of four chromosomes. Further, nothing is yet known of the possible relations of **nx** to any of these genes. It would be difficult, however, to deny the suggestive trend of this genetical evidence. Clearly, autotetraploidy does offer a sound working hypothesis on which to interpret potato genetics, and, so far as they go, the existing

data supply a very good indication that the potato is, in fact, an autotetraploid.

As regards the immediate problem in hand, there should now be no difficulty in synthesizing plants top necrotic to virus *X*. For breeding purposes the quadruplex **Nx** individuals recoverable from simplex and duplex selfed families will be the most generally useful, since all the gametes from such plants will carry the dominant allele.

Consideration of viruses *A*, *B* and *C* will be reserved for a later contribution. It is, however, possible to say that the inheritance of the top-necrotic reaction to virus *A* is controlled by a dominant gene **Na** closely linked to **Nx**. Both coupling and repulsion phases of this linkage are distinguishable, as is expected in autotetraploids. So far the virus *B* and *C* reactions have shown only disomic ratios.

Finally, there is one point worth noting in connexion with autotetraploid segregations. The amount of double reduction at any locus is a function of the cross-over distance between that locus and the centromere. Hence it follows that 'there is no segregation characteristic of an autotetraploid, but each gene will have its own segregation which may not be constant since crossing-over is affected by environment' (Mather, 1936).

In the past, random chromosome (Muller, 1914) and random chromatid (Haldane, 1930; Sansome, 1933) segregations have been regarded as the limiting types of autotetraploid behaviour. The first will be expected when separation at a locus is completely reductional. Random chromatid segregation presupposes infinite crossing-over and completely random disjunction of the chromosomes of a multivalent. Since neither of these conditions is actually fulfilled, the usefulness of the random chromatid hypothesis is very limited. As Mather (1935*a*) has shown ratios approaching those expected on the random chromatid basis should be considered as due to a combination of the two types of separation, equational and reductional in the random proportions of $\frac{6}{7}:\frac{1}{7}$ respectively at the locus concerned.

Summary

In the face of cytological evidence to the contrary, the cultivated potato, with $2n=48$ chromosomes, has been regarded by geneticists as a functional diploid. All the available evidence indicates that it does in fact behave as an autotetraploid.

A major difficulty in dealing with polyploid material is to recognize phenotypic differences associated with the segregation of individual

genes. In the present case it has been found that individual potato varieties may respond with one of two reactions to graft infection with either of viruses *X*, *A*, *B* or *C*. Either (*a*) a lethal necrosis develops which eventually kills the whole plant, or (*b*) the foliage develops a mosaic of varying degrees of intensity. Data have been presented to show that the necrotic reaction to virus *X* infection is associated with the dominant allele of a gene designated **nx**.

The segregation of this gene has been followed in crosses between commercial potato varieties and proves to be tetrasomic. Most necrotic-reacting varieties are simplex for the dominant allele; so far only one duplex type has been found. The two phases of dominance are indistinguishable phenotypically.

The four chromosomes concerned pair and cross-over at random and must therefore be extensively if not completely homologous. This is deduced from (*a*) the occurrence of double reduction at the **nx** locus, and (*b*) the evidence of partner exchange provided by the simplex and duplex indices of separation for this gene. It appears that the **nx** gene is located some distance from the centromere.

Consideration of these findings in conjunction with recent genetical work shows that autotetraploidy offers a sound working hypothesis for further investigations.

These conclusions are also of immediate practical significance. It has been shown that plants reacting with lethal necrosis to graft infection with virus *X* are virtually immune to this virus under field conditions. There should now be no difficulty in synthesizing breeding stocks quadruplex for the **Nx** allele and, hence, in producing economically useful plants field immune to virus *X*.

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EXPLANATION OF PLATE 2

Fig. 1. Early stages of virus *X* top necrosis in Edgecote Purple. The apical leaves are beginning to wither and dry up and necrotic streaks can be seen spreading down the stems.

Fig. 2. A leaf from a seedling showing local necrosis following inoculation by rubbing with a sap extract from virus *X*-infected Arran Victory.

Fig. 3. The recessive type; a leaf from a virus *X*-infected plant of Arran Victory showing a mild mosaic.

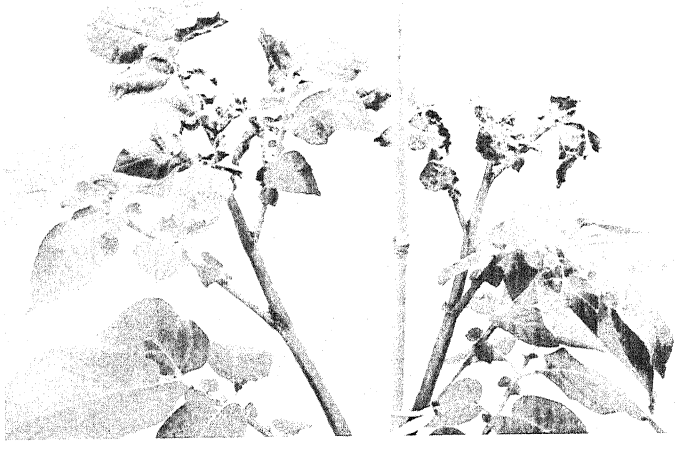

Fig. 1.

Fig. 2.

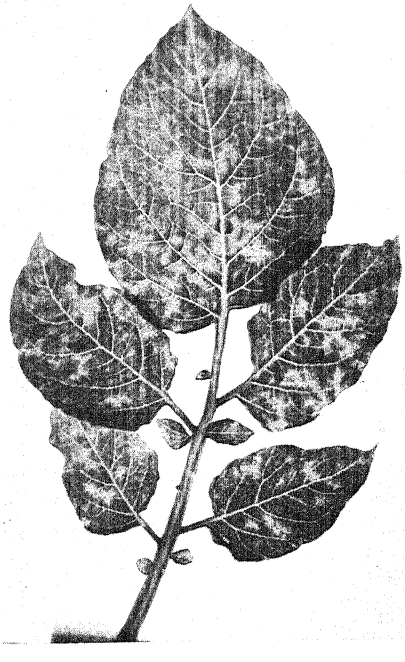

Fig. 3.

X-RAY-INDUCED STRUCTURAL CHANGES IN THE CHROMOSOMES OF *DROSOPHILA PSEUDO-OBSCURA*

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(With One Text-figure)

Introduction

Drosophila pseudo-obscura differs fundamentally in its chromosome morphology from *D. melanogaster*. While the latter has a rod-shaped *X*, two V-shaped autosomes and a small dot chromosome, in *D. pseudo-obscura* the chromosome make-up consists of a V-shaped *X*, three rod-shaped autosomes of various sizes, and one small dot-chromosome. The *Y*-chromosome is similar in both species. Genetical analysis by Crew & Lamy (1935), Donald (1936), Sturtevant & Tan (1937), and Sturtevant & Novitski (1941) has shown that in spite of the profound morphological and genetical differences the various chromosomes, or arms of chromosomes, in these two species are related phylogenetically.

Several cytogenetical studies analysing the type and frequency of X-ray-induced chromosome rearrangements in the salivary gland nuclei have been carried out on *D. melanogaster* (Bauer, Demerec & Kaufmann, 1938; Catcheside, 1938; Bauer, 1939; Kaufmann, 1939). The information which has been collected on irradiated *melanogaster* has thrown light upon various problems concerning chromosome structure and behaviour. We can visualize now the possible mechanism by which structural changes are induced in the chromosomes when exposed to X-rays. In view of the fact that *melanogaster* and *pseudo-obscura* differ in their chromosome morphology and constitution it seemed desirable to carry out similar investigations in *pseudo-obscura* in order to ascertain how far the conclusions arrived at in *melanogaster* can be applied to a different organism.

While these experiments were in progress a paper was published by Helfer (1941) dealing with the X-ray-induced chromosomal variations in *D. pseudo-obscura*. His data give an opportunity of comparing two sets of data for *D. pseudo-obscura* and of relating them to those found in similar experiments in *D. melanogaster*.

Material and methods

A highly inbred *D. pseudo-obscura* Texas strain was used throughout the experiments. This strain belongs to race A, and its gene arrangement is designated as the 'standard' (Dobzhansky & Sturtevant, 1938). Two- to ten-day-old male flies were irradiated. The dosage was measured during the radiation by a Siemens integrating Dosimeter and it was kept constant (4500 r. units) throughout the experiments; the radiation was obtained by using 80 kV. 6 mA., 0·5 mm. aluminium filter, 25 r./m. Treated males were mated, immediately after X-raying, to females of the same strain; fourteen males and fourteen females were placed together in the same vial. The average age of females at the time of mating was 10 days. The larvae were grown at about 15–16° C. In order to avoid overcrowding of the larvae the following procedure was followed: X-rayed males and untreated females were kept in vial cultures together for 2 days, afterwards being transferred into ½ pint culture bottles for 3 days. The females were then separated from the males and were transferred three times into new culture bottles at intervals of 3 days.

By raising three broods we were able also to obtain a larger number of female larvae for cytological analysis because these came to maturity in an appreciably shorter time than the males and were easy to select in cultures which are not overcrowded. This selection was necessitated by the fact that in the nuclei of the salivary glands an interchange between the *Y* and an autosome cannot readily be distinguished from one between two autosomes when one of the breaks in the latter has occurred in the heterochromatic region.

The structural changes were analysed in the chromosomes of the salivary gland nuclei of F_1 female larvae. The breakage points were determined within the limit of at least one subdivision of the map constructed by Dobzhansky & Sturtevant (1938) for the third chromosome; in the other chromosomes only the number of breaks and the type of rearrangement was studied.

Number of breaks

The salivary gland chromosomes of 154 F_1 larvae out of the total of 425 analysed have shown new rearrangements. The total number of breaks in 2125 chromosomes was found to be 518. This number represents only the residue of total breaks induced by X-raying; the initial number of breaks is probably much higher. It is known that many ionizations do not produce breaks because they do not occur in the proper atoms

(Sax, 1939, 1940; Muller, 1940*a*); and many breaks undergo restitution (Faberge, 1940; Sax, 1940; Darlington & Upcott, 1941). A great number of breaks which give rise to new chromosome rearrangements cannot be detected because they involve the heterochromatic region only, and another portion leads to dominant lethal rearrangements (such as deletions, aneucentric interchanges and sister chromatid reunions). At present we have no means of determining the frequency of those breaks which are either eliminated as dominant lethals or undetectable owing to restitution.

Helfer (1941) in *D. pseudo-obscura*, Bauer *et al.* (1938), Catcheside (1938) and Bauer (1939) in *D. melanogaster*, have made a similar study of the effect of X-rays upon the chromosomes. The data obtained by these investigators and by ourselves are given in Table 1.

Table 1. *A comparison of the number of sperms in* D. melanogaster *and* D. pseudo-obscura *showing induced chromosome rearrangements*

	D. melanogaster					*D. pseudo-obscura*	
	Bauer *et al.* (1938)		Bauer (1939)		Catcheside (1938)	Helfer (1941)	Koller & Ahmed
X-ray dosage, in r. units ...	4000	5000	4000	5000	4000	5000	4500
No. of larvae examined	215	217	447	608	224	413	425
No. of changed sperms	64	87	144	273	73	132	170
% of sperms with chromosomal rearrangements	29·8 ± 3·12	40·0 ± 3·33	32·21 ± 2·21	44·90 ± 2·02	32·6	32·0	40·00 ± 2·23
No. of breaks	184	272	427	376	172	347	518
% of breaks per changed sperms	287·5	312·6	296·5	320·8	237·0	263·0	304·7
% of breaks per total sperms	85·58	125·34	95·25	144·07	81·20	84·02	121·76

The frequency of surviving larvae carrying chromosome rearrangements, when the difference in dosage is taken into account, is approximately the same in *melanogaster* and *pseudo-obscura*. According to the data of Bauer *et al.* (1938) a dosage of 4500 r. units is expected to induce structural changes in 38·5% of the sperms treated. Our data are in close agreement with those of Bauer *et al.*, Bauer, and Catcheside, but there is a significant difference between ours and Helfer's. The discrepancy may be due either to an error in determining the dosage used by Helfer, to his failure to identify small chromosome rearrangements, or to a misinterpretation of some reunions. It is not improbable, however, that the cause of the discrepancy may lie entirely in the different environmental conditions under which his and our experiments were carried out.

A comparison of the data concerning the frequency of breaks per detectable changed sperm in *melanogaster* and *pseudo-obscura* indicates that the same amount of ionization apparently induces the same number

of breaks in the chromosomes of *pseudo-obscura* as in those of *melanogaster*. Since the total chromosome length is approximately the same in *melanogaster* and *pseudo-obscura* (Sturtevant & Novitski, 1941), and the number of chromosome arms is six in the sperm as well as in the salivary

Table 2. *Distribution of chromosome breaks in X-rayed sperm*

Species	Author	Dosage in r.	No. of breaks 0	2	3	4	5	6	7	Undeterminable	Total
D. melanogaster	Bauer *et al.*	5000	130	47	9	14	9	2	4	1	216
D. pseudo-obscura	Helfer	5000	281	92	10	19	9	2	—	—	413
D. pseudo-obscura	Koller & Ahmed	4500	255	100	8	36	9	14	3	—	425

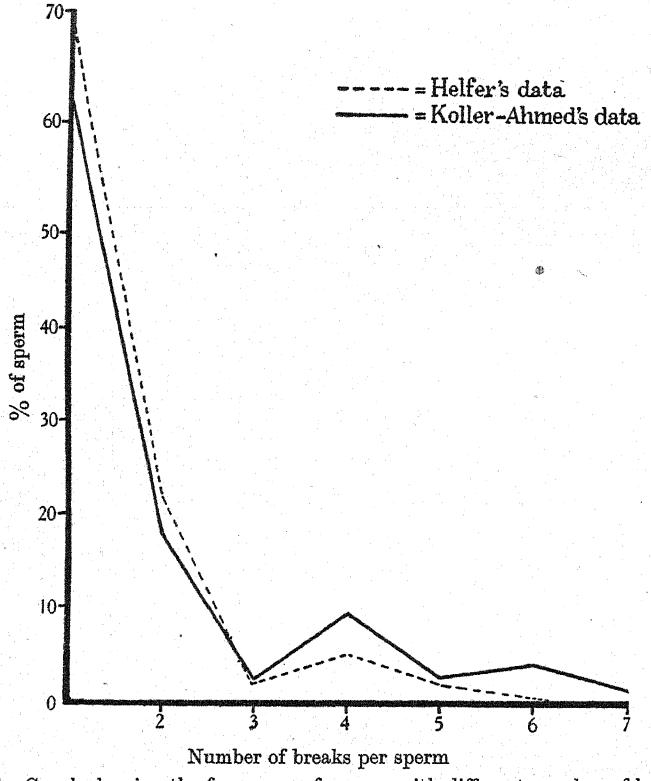

Fig. 1. Graph showing the frequency of sperms with different number of breaks.

gland nuclei of both species, we may conclude that there is no genetic difference in respect of the reactivity of chromosomes to X-rays in these two species.

The distribution of chromosome breaks in the sperms is given in Table 2 and illustrated in Fig. 1. It can be seen that one-break cases,

i.e. single terminal deficiencies, are absent. The mechanism which leads to the elimination of chromosomes with broken ends is discussed by McClintock (1939), Muller (1940*a*), Pontecorvo (1941), and Darlington & Upcott (1941). They assume that either a fusion of sister chromatids or a non-division of chromomere (or gene) at the broken end could result in the formation of a dicentric which is eliminated in successive cell generations. 'Healing' of broken ends, which means the origination de novo of a new telomere at the broken end in the chromosomes of *Drosophila* is very rare, if possible, according to Muller (1940*a*). There is evidence in maize that while the cycle of bridge-breakage-fusion-bridge can apparently continue uninterruptedly in the endosperm the broken end becomes healed in the embryo and plant tissues (McClintock, 1939, 1941). Instances are also reported which seem to indicate that telomeres, i.e. existing chromosome ends, are not necessarily permanent structures in *Drosophila*. Thus Demerec & Hoover (1936) have described terminal deficiencies in *D. melanogaster*, and recently five more cases have been reported by Sutton (1940). In *D. ananassae*, Kaufmann (1936) identified terminal inversions. It must, however, be pointed out that telomeres are heterochromatic in nature as is indicated by their non-specificity (Bauer, 1936). In the salivary gland nuclei it is known now that such structures are difficult to analyse, therefore we may consider that a more definite proof is still needed as to whether or not terminal deficiency can exist in *Drosophila*. If it does, the frequency must be very small.

The other interesting fact is indicated by the relatively low frequency of sperms with chromosome rearrangements which require 3, 5, and 7 breaks. The low frequency one may assume to be due to the sister chromatid reunions, which are necessarily produced in the presence of an uneven number of breaks. When the breaks are distributed in different chromosomes, or chromosome arms, it is expected that an uneven number of breaks can lead to viable rearrangements only when deletion-insertion, or pericentric and intra-arm inversion takes place. Such a rearrangement is very rare as compared with other types. A further analysis of the relative frequency of breaks in the same chromosome arms and in the two arms of the *X*-chromosome has shown that the scarcity of uneven numbers does not hold true for intra-arm rearrangements (Tables 3 and 4). The fact that the odd break in inter-arm rearrangements disappears can be explained by assuming that the odd break either (1) undergoes restitution, or (2) sister chromatid reunion, which is followed by bridge formation and elimination of the dicentric chromosome. The data, though very meagre, suggest that the 'terminal deficiency' effect is probably not the

real and only cause of this scarcity; it may be due to a 'proximity effect' (Muller, 1940*a*), or 'competition' (Darlington & Upcott, 1941), which favours the restitution of the odd break.

Table 3. *Frequency of breaks in the same chromosome arm*

No. of breaks	2	3	4	5	6	Total
No. of cases	97	20	8	—	1	292

Table 4. *Frequency of breaks in the two arms of X-chromosome*

Chromosome arm	No. of breaks 1	2	3	4	5	Total
XR	19	5*+ 7	6 (1)†	1	—	65
XL	10	5*+ 5	4 (2)	1	—	46
Total	29	10*+12	10 (3)	2	—	111

* Inversion. † Number in ( ) indicates inversion.

Frequency of breaks in three broods

With the aim of obtaining some additional data on the problem of whether or not there is a reduction in the frequency of chromosome rearrangements when the time interval between treatment and the mating is increased, an analysis of chromosomes in the salivary gland nuclei of two more broods of the same treated males was undertaken. Brood II was obtained by mating 15-day-old males to virgin females. The same males were previously kept together with females, their offspring constituting brood I. Brood III consists of larvae obtained by mating the same males a third time to virgin females, the males being then 20 days old. It can be seen that the method used by us is very similar to that employed by Patterson (1933). While, however, the time between X-raying and mating varied from 1 to 10 days in *melanogaster*, in our experiment the period has been 0–5, 6–10 and 11–15 days respectively. The results obtained are given in Table 5.

Table 5. *Distribution of chromosome rearrangements and breaks among the different broods*

Age of X-rayed males at mating	No. of sperms examined	No. of changed sperms	% of changed sperms	No. of total breaks	Breaks per changed sperms
First brood (10–15 days old), 0–5 days from X-raying	34	18	52·93 ±	51	2·8
Second brood (15–20 days old), 5–10 days from X-raying	150	67	44·42 ±	199	2·8
Third brood (20–25 days old), 10–15 days after X-raying	241	85	35·26 ±	268	3·15

It can be seen that the frequency of sperms with chromosome rearrangements differs somewhat in the three broods. The difference is not significant, P being 0·6. The small number of larvae examined in the first brood is known to be due to the temporary arrest of the mating instinct produced by irradiation in the male parent. It was observed that within 5 days after treatment the mating instinct of X-rayed males was absent, and that only a few of the fourteen treated males had mated. No such suppression of mating instinct has been observed in *melanogaster*. Patterson found a period of great infertility extending from the fourth day throughout to the tenth day. He concluded that in the males the immature germ cells were killed by X-rays. It is more probable, however, that their division was suppressed. According to Demerec & Kaufmann (1941) the number of sperms fully matured and available for immediate transfer is sufficiently limited and they are exhausted in a few consecutive matings. In *D. melanogaster*, sperms which were immature during X-ray treatment are ready to be used between the twelfth and nineteenth day, provided the old sperms are used up. Our data suggest either that in 10-days-old, or older, *pseudo-obscura* most of the germ cells, if not all, have undergone already spermateleosis, or that the sperms used within 15 days after the irradiation are fully matured at the time of X-ray treatment. While in *melanogaster* there is also, according to Patterson, a differential effect of X-rays for certain stages of the germ cells, no such effect was detected in *pseudo-obscura* males whose age was over 10 days at the time of treatment. Further experiments have been planned to extend the limit and to increase the number of periods with a view to obtaining data concerning the frequency of chromosome rearrangements induced in the germ cells during the meiotic and premeiotic stages.

FREQUENCY OF DIFFERENT TYPES OF REUNION

Various difficulties may be encountered in the interpretation of some complex chromosome rearrangements. Such is the case when analysing structural changes which involve breaks in the different arms of the same chromosomes. Catcheside (1938) calls such breaks, when followed by interchanges between the arms, 'eucentric' (pericentric, according to the terminology of Muller, 1940*b*) inversions. In view of the fact that in the nuclei of the salivary glands, as well as in the nuclei of sperms, the chromosome arms are considered as independent units, and behave as such in their reaction to X-rays, we classified these 'eucentric' in-

versions of Catheside as interchanges, though we admit that from a genetical point of view they are not true interchanges.

The multiple chromosome rearrangement which is described by Helfer as a 'terminal intercalation' may be interpreted as three interchanges involving three chromosomes and a duplication. Helfer has described a 'branched' chromosome. No such structural change was found in our experiments. Hitherto, the only cases of branched chromosome have been reported by Darlington (1929) in *Tradescantia* and by Levan in *Allium* (1932), and by Mather & Stone (1933) in *Crocus*. Kossikov & Muller (1935) have presented critical proof that in the case of Pale translocation in *Drosophila melanogaster* the fragment from the second chromosome which was postulated by Morgan, Bridges & Schultz (1932) as attached to the third chromosome laterally, became inserted within the chromosome without any disturbance in the linear arrangement of genes.

During our experiments 364 chromosome rearrangements were identified in 154 sperms. The frequency of the various types is as follows:

Interchanges	218
Inversion	143
Duplication	1
Deficiency	1
Shift (intra-chromosomal translocation) ...	1

The duplication encountered during the experiment consists of the presence of two identical segments lying adjacent to each other in an inverted sequence. We postulate two breaks in explaining the origin of this particular duplication. Kaufmann & Bate (1938) believe that rearrangements of this type may be the result of three independent breaks instead of two. According to them, one of the breaks (*a*) occurred in one sister chromatid and the two others ((*b*) and (*c*)) in the other chromatid. They assume that breaks (*a*) and (*b*) occurred at identical loci. If the breaks are not at identical loci a haploid loop should be present at the end of the paired, duplicated segment. The absence of such configuration in our material indicates that the breaks occurred at identical loci. Muller (1940*a*) postulates that the extreme exactitude of correspondence between points of breakage and reunion of the two species constituting the duplication furnishes a virtual proof that these breaks must have occurred before the chromosome divided into two sister chromatids. He argues that the chance of another independent ionization taking place at an exactly corresponding locus in the sister chromatid is negligibly small. According to Catcheside (unpublished) the chance that a break

occurs in the sister chromatid at the same corresponding locus is much greater when the ionization belongs to the same track of ionization produced by the same ionizing electron. While Delbrück (1940) has shown that the possibility of one ionization breaking two sister chromatids must be excluded on physical grounds, Catcheside and co-workers (unpublished) have obtained proof in *Tradescantia* that one ionization alone has a small chance of breaking one chromosome, hence the chance of breaking two chromatids is even much smaller. We assume that breaks responsible for duplication in *Drosophila*, which might have been produced by one or more ionizations, occurred before chromosome division, and that reunion took place after the chromosome had divided into two chromatids.

Our data also show that the frequency of deficiencies is very low. While it is not improbable that a number of small deficiencies, which consist in the loss of only a few bands in the salivary gland chromosome, were overlooked during the analysis, large deficiencies could easily be identified by the presence of haploid loops. The failure of detection of large deficiencies indicates that such losses either do not occur in the autosomes, or if they do, they must have been eliminated in the zygote. It is reasonable to assume that the absence of deficiencies in the F_1 generation of X-rayed males is due to elimination rather than to non-occurrence. It must also be pointed out that a large deficiency in the X-chromosome may lead to the transformation of female into male; such a change could not be detected in our experiment owing to the fact that only female larvae were analysed.

The frequency of the various rearrangements is compared in Table 6. The data obtained in *pseudo-obscura*, as well as in *melanogaster*, show that

Table 6. *The frequency of intra-arm and inter-arm rearrangements in two and in multiple break combinations*

Chromosome	2 breaks			3–7 breaks			Total		
	Intra-*	Inter-†	Total	Intra-	Inter-	Total	Intra-	Inter-	Total
XR	5	7	12	12	18	30	17	25	42
XL	7	5	12	5	18	23	12	23	35
II	6	16	22	31	38	69	37	54	91
III	8	10	18	12	31	43	20	41	61
IV	20	15	35	37	59	96	57	77	134
V	—	1	1	—	—	—	—	1	1
Total	46	54	100	97	164	261	143	221	364

* Intra-arm rearrangement = inversion.
† Inter-arm rearrangement = interchange (translocation).

the frequency of inversions is higher, and that of the interchanges is lower than expected on the assumption that (1) the chromosome arms

are of the same length, (2) every chromosome has the same breakability, and (3) breaks occur at random. According to the formula of Bauer *et al.* which is based upon the above assumptions, the expected proportions of interchanges and inversions is 4 : 1. Our data show that there is a significant discrepancy between the observed and expected frequency of intra- and inter-arm rearrangements. Similar data have been obtained by Catcheside (1938) according to which he suggested that reunion of broken ends is at random rather than the occurrence of breaks. The explanation of the discrepancy is found in the fact that on purely mechanical grounds the survival of an inversion in successive cell generations is much higher than that of an interchange, which can be of 'aneucentric' type (Darlington, 1939; Darlington & Upcott, 1941).

According to the extensive genetical analysis of Muller (1940*a*), the 'proximity effect' necessarily favours the reunion of broken chromosomes to form inversions at the expense of interchanges. This effect is great enough to account for the discrepancy already existing between the expected and observed proportion of inversions and interchanges.

Another source of error which reduces the frequency of inter-arm rearrangements lies in the structural peculiarities of the heterochromatic region of the salivary gland chromosomes. The breakage frequency of this region is as high as that of the euchromatic region if chromosome length at somatic metaphase is taken into consideration (Kaufmann, 1939; Bauer *et al.* 1938). The frequency of interchanges being higher than that of inversions rearrangements in the heterochromatic region necessarily alter the proportion in favour of the inversions because structural changes in this segment cannot always be detected cytologically.

Distribution of breaks in the different chromosomes

The number of breaks was determined in each of the six chromosome arms. Their distribution amongst the various chromosomes is given in Table 7. Apparently the small, dot-like chromosome (fifth) has the lowest frequency, but when the frequencies are calculated per unit of length of the various chromosomes it was found that the breakage frequency of the fifth autosome is nearly the same as that of the second and third chromosomes. When our data are compared with those of Helfer, it can be seen that there are small discrepancies in respect of (i) chromosome length, and (ii) breakage frequency.

(i) Presumably as a consequence of the small size the error of any measurement is relatively large. The approximate lengths of the chromo-

somes in *D. pseudo-obscura* during mitotic metaphase as measured by Koller (1936) are given as follows:

X	2·8 μ	4th	1·0–1·25 μ
2nd	1·25 μ	5th	(not given)
3rd	1·25 μ		

At mitotic metaphase the second, third and fourth chromosomes appear as single rods, all of them having a subterminal centromere. The short arms of these chromosomes probably consist of a very few genes.

Table 7. *Break percentage and mitotic chromosome length in* D. melanogaster *and* D. pseudo-obscura

D. pseudo-obscura							*D. melanogaster*			
	Koller & Ahmed			Helfer*				Bauer *et al.*		
Chromosome	No. of breaks	% of breaks	% of total chromosome length	No. of breaks	% of breaks	% of total chromosome length	Chromosome	No. of breaks	% of breaks	% of total chromosome length
XR	65	11·7	22·6	40	21·4	24·9	III *L*	161	20·7 ±1·5	18·3
XL	46	9·0	13·9	19	10·2	12·4	*X*	158	20·4 ±1·5	18·7
II	153	29·8	27·1	48	25·7	24·9	III *R*	146	18·8 ±1·4	23·7
III	79	15·4	19·8	31	16·6	17·3	II *R*	158	20·4 ±1·5	20·1
IV	174	33·9	16·7	43	23·0	19·8	II *L*	142	18·3 ±1·4	17·8
V	1	0·2	0·9	6	3·2	1·2	IV	11	1·4 –0·4	1·4

* Only ♀♀ larvae were considered.

The long arms are approximately equal in length in the three autosomes. Both arms of the *X*-chromosome are slightly longer than the rod-shaped autosomes. In the salivary gland nuclei the relative lengths of the various chromosome arms differ and the differences are due to the different lengths of the heterochromatic segments lying next to the centromere of the various chromosomes. It was found that *XL* has twice as long a heterochromatic region as the *XR*, reducing the ratio between the euchromatic parts of *XR* and *XL* from 1 : 1 to 2 : 1 according to Helfer (1941). Tan (1935), on the other hand, stated that the length of *XL* is two-thirds that of *XR*, and Koller (1936) reported that the length of the two arms of *X* together is almost equal to the total length of the second, third and fourth autosomes together. According to Dobzhansky & Tan (1936) the *XR* is the longest element in the chromosome complement of the salivary gland nuclei. These discrepancies in the data given by the various authors are attributable to the different methods of handling the smear preparations in which salivary gland chromosomes are measured.

In order to make a comparison of chromosome length and number of breaks more feasible the salivary gland chromosomes were measured in

forty fully grown female larvae, the measurements being made on the most outstretched chromosomes, and the highest figure obtained was taken to represent the length of a particular chromosome. For convenience the total length of the chromosome make-up in the salivary gland nuclei is represented by 100 units, and the relative lengths of the various chromosomes in units is as follows:

XL	13·9	2nd	27·1	4th	16·7
XR	22·7	3rd	19·8	5th	0·9

The ratios of the lengths of the euchromatic portions of the different chromosomes are given by Helfer (1941) as follows:

XL	5	2nd	10	4th	8
XR	10	3rd	7	5th	0·5

It can be seen that Helfer's data are comparable with ours, the differences being not significant.

(ii) However, when the breakage frequencies of the different chromosomes are considered, and the data obtained by Helfer in *pseudo-obscura* and Bauer *et al.* in *melanogaster* are compared with ours, it becomes obvious that *XR* apparently has significantly lower, and the fourth chromosome a higher number of breaks per unit chromosome length. It was observed that in *melanogaster* the distribution of breaks in the euchromatic sections of chromosomes is directly proportional to chromosome length (Bauer *et al.* 1938). Helfer also stated that in *pseudo-obscura* the X-ray-induced breaks are distributed amongst the chromosomes 'more or less' in proportion to their length. Our data, however, suggest that breakability of chromosomes may differ, and breaks can accumulate in one particular chromosome. The high frequency of X-ray-induced breaks in the fourth chromosome one may contrast with the high frequency of spontaneous breaks in the third chromosome. In natural population of *D. pseudo-obscura*, Dobzhansky & Sturtevant (1938) found that the third chromosome has seventeen different gene arrangements consisting of inversions. These structural changes were brought about by thirty-eight independent breaks in the third chromosome, while very few breaks, or no breaks at all, were found in the other chromosomes. Sturtevant & Mather (1938) have attempted to explain the accumulation of inversions in the third chromosome of *D. pseudo-obscura* and argue that heterosis and selective advantage are responsible for this phenomenon. At present no adequate explanation can be given as to why the frequency of X-ray-induced breaks is disproportionately high in the fourth chromosome and low in *XR*.

According to various investigators (Donald, 1936; Sturtevant & Tan, 1937; Dobzhansky & Sturtevant, 1938; Sturtevant, 1938) the following relationship was identified between the chromosomes of *melanogaster* and *pseudo-obscura*:

$X^m : XL^p$	$3L^m : XR^p$
$2L^m : 4^p$	$3R^m : 2^p$
$2R^m : 3^p$	$4^m : 5^p$

(m stands for *melanogaster* and p for *pseudo-obscura*).

It can be seen that $2L$ and $3L$ of *melanogaster* correspond to the fourth and XR of *pseudo-obscura* respectively, hence one might perhaps expect a higher number of breaks per unit chromosome length in $2L$ and a lower number in $3L$ of *melanogaster*. Our data suggest that these particular chromosome arms have a different breakability than the other chromosome arms in *pseudo-obscura* and its corresponding partners in the *melanogaster* genotype.

THE DISTRIBUTION OF MULTIPLE BREAKS IN THE CHROMOSOMES OF *MELANOGASTER* AND *PSEUDO-OBSCURA*

There are difficulties of various kinds when we attempt to construct a table showing the frequency and the types of multiple breaks in the two species. When comparing the data it must be kept in view that they are obtained under different conditions. First of all the figures, as they are given by Bauer *et al.*, are derived from experiments in which the dosage varied from 1000 to 5000 r. units. Helfer's and our data were obtained by using one dosage only (5000 and 4500 r. units respectively). Helfer analysed male and female larvae and therefore encountered rearrangements which necessarily involved the Y-chromosome: in our experiments only female larvae were chosen for analysis.

The data concerning the distribution of multiple breaks amongst the chromosome arms in *melanogaster* and *pseudo-obscura* are summarized in Table 8. The distribution and frequency of two breaks have already been discussed, and the causes underlying the discrepancies between the observed and expected proportion of interchanges and inversions have been debated. A similar observation can be made in respect of multiple breaks. The possible distribution of 3 breaks is 1–1–1; 2–1 or 3, and our data show that the 2–1 break distribution is the most frequent. The expected frequency of each class is 48%–48%–4%, according to the formula given by Bauer *et al.* Our data, as well as similar data obtained in *melanogaster*, clearly show that there is a significant discrepancy between

the observed and expected frequencies. A mathematical calculation of Pontecorvo (unpublished) indicated that the 'expected' frequencies of Bauer *et al.* are wrong because the various proportions of dominant lethal recombinations in inter- and intra-arm rearrangements were not taken into consideration. Our data suggest also that breaks in the same chromosome arm are favoured in the formation of new rearrangements instead

Table 8. *Distribution of multiple breaks among the chromosome arms*

		Chromosome rearrangements					
		Number			%		
		D. melanogaster	*D. pseudo-obscura*		*D. melanogaster*	*D. pseudo-obscura*	
No. of breaks	Types and distribution	Bauer *et al.*	Helfer*	Koller	Bauer *et al.*	Helfer*	Koller & Ahmed
2	1, 1	97	35	54	61·78	63·64	54·00
	2	60	20	46	38·22	36·36	46·00
3	1, 1, 1	2	2	1	7·41	66·67	12·50
	2, 1	20	0	6	74·07	—	75·04
	3	5	1	1	18·52	33·33	12·50
4	1, 1, 1, 1	19	4	10	47·50	36·36	27·78
	2, 1, 1	6	3	2	15·00	27·27	5·55
	3, 1	6	3	7	15·00	27·27	19·44
	2, 2	9	1	13	22·50	9·09	36·11
	4	0	0	4	—	—	11·11
5	1, 1, 1, 1, 1	0	0	1	Owing to the low frequency of 5, 6, 7 breaks the percentage was not calculated		
	2, 1, 1, 1	2	2	2			
	2, 2, 1	4	0	1			
	3, 1, 1	0	1	0			
	3, 2	6	1	4			
	4, 1	0	1	1			
6	2, 2, 1, 1	5	0	3			
	3, 1, 1, 1	0	0	1			
	3, 2, 1	0	0	3			
	4, 1, 1	1	0	0			
	3, 3	0	0	3			
	4, 2	0	0	3			
	6	0	0	1			
7	1, 1, 1, 2, 2	0	0	1			
	1, 1, 2, 3	1	0	0			
	2, 2, 2, 1	0	0	1			
	2, 2, 3	1	0	1			

* Only female larvae are considered.

of undergoing restitution. The data obtained by Helfer in *pseudo-obscura*, however, when the distribution of breaks is considered only in the female larvae, indicate an unexpected chromosome behaviour. He found that the number of chromosome rearrangements in which the breaks are distributed in different chromosomes or chromosome arms is higher than those in which the breaks are accumulated in the same chromosome. This finding of Helfer is the more striking because all the data so far reported in the literature concerning the distribution of X-ray induced

breaks in *Drosophila* indicate that breaks are accumulated in the chromosomes rather than being distributed at random. Kaufmann (1938) analysed a case in which five out of the ten breaks occurred in one chromosome arm. In our experiment four sperms were recovered in which all four breaks occurred in one chromosome. A similar case was found where the number of breaks is six, and all the breaks occurred in the fourth chromosome, giving rise to three independent inversions.

The distribution of multiple breaks shows that the combination of break loci is restricted. Breaks in the chromosomes lead to independent rearrangements rather than to simultaneous exchange among all the altered chromosomes. The high frequency of exchanges in the same chromosome arm as compared with inter-arm exchanges is another point in favour of the breakage-first hypothesis (Stadler, 1932; Muller, 1940*a*, Bauer *et al.* 1938). The close agreement between the data obtained by Bauer *et al.* in *melanogaster* and ours in *pseudo-obscura* indicates that the mechanism involved in producing breaks and reunions in the chromosomes is similar in these two species. The data reported by Helfer, when the break distribution in both sexes is taken into consideration, show a close agreement with ours. The discrepancy observed by him in the two sexes is based upon a small number of cases, and is not sufficient to alter our concept of the mechanism by which new rearrangements are produced in the chromosomes of *Drosophila*.

DISTRIBUTION OF SPONTANEOUS AND X-RAY-INDUCED BREAKS ALONG THE THIRD CHROMOSOME OF *D. PSEUDO-OBSCURA*

Dobzhansky & Sturtevant (1938) constructed a detailed cytological map of the third chromosome which enables us to determine more accurately the loci of breaks and to compare the distribution of the X-ray-induced ones with that of the spontaneous breaks. In different wild populations of *D. pseudo-obscura*, Dobzhansky & Sturtevant (1938) have found seventeen structural arrangements of the third chromosome. The total number of breaks (as disclosed by the analysis of the various band-sequences) was found to be thirty-nine. The number of X-ray-induced breaks located in the third chromosome in Helfer's experiment was seventy-one, while in ours it is sixty-five (excluding fourteen breaks which could not be localized accurately). The loci and frequencies of various breaks are given in Table 9.

The third chromosome, according to the map of Dobzhansky & Sturtevant, is divided into nineteen sections of more or less similar size. These divisions are numbered from 63 to 81; the former is the most

proximal, adjacent to the heterochromatic region, the latter is the segment which includes the tip (or telomere) of the chromosome. In constructing the table we followed Helfer's system, i.e. a break found near or at the boundary between two divisions was considered as half of a break belonging to each of them.

A comparison of the distribution of induced and spontaneous breaks shows clearly that while the heterochromatic region exhibits a high breakability to X-ray radiation, or a higher survival value of the rearrangement, including a break in it, or less restitution for each break,

Table 9. *Distribution of natural and induced breaks in the third chromosome*

Divisions according to Dobzhansky & Sturtevant (1938)	No. of cases			Percentage of cases		
		Induced breaks			Induced breaks	
	Natural	Helfer	Koller & Ahmed	Natural	Helfer	Koller & Ahmed
Heterochromatic region	0	10	8	0	13·7	12·3
63	0	0	1	0	0	1·6
64	1	2	4	2·6	2·8	6·2
65	1	1	4	2·6	1·4	6·2
66	0	7	1	0	9·6	1·6
67	0	2	5	0	2·8	7·1
68	2	2	4	5·3	2·8	6·2
69	3	4	0	7·9	5·5	0
70	4	7	6	10·5	9·6	9·2
71	3	1	3	7·9	2·8	4·6
72	1	5·5	4	2·6	7·5	6·2
73	0·5	2·5	3	1·3	3·4	4·6
74	1·5	1	1	4·0	1·4	1·6
75	2	6·5	7	5·3	8·9	10·8
76	5	1	3	13·2	2·8	4·6
77	2·5	4·0	3	6·6	5·5	4·6
78	2·5	3·5	4·5	6·6	4·88	6·9
79	5·5	7	2·5	14·5	9·3	3·9
80	1·5	4	0	4·0	5·5	0
81	2	2	1	5·3	2·8	1·6

not one spontaneous break occurred in this segment. In the euchromatic part division 70 contains a high number of spontaneous as well as induced breaks. In section 75, on the other hand, only the X-ray-induced breaks are numerous. Already Tan (1937), and Dobzhansky & Sturtevant (1938) have drawn attention to the fact that natural breaks are accumulated in restricted regions of the third chromosome. From their data and ours it appears that the spontaneous and X-ray-induced breakage points of inversions are not distributed at random, e.g. several natural breaks were localized in the short sections of 76–77, while in the proximal region from 63 to 67, which constitutes more than one-third of the total length

of the chromosome, only two natural breaks were found. When Helfer's and our data are considered together and analysed statistically by the χ^2 method, it is found that while the distribution of X-ray-induced breaks is not random, that of the spontaneous breaks is. The evidence of non-random distribution is, however, not very strong.

It should be pointed out that natural breaks differ from induced breaks in one important respect. They must not only be able to survive mechanically as the induced breaks must, but they have also been in existence in the wild for a long time and so must selectively be sound. This does not apply to the induced breaks, and it is possible that any differences in distribution might be related to this fact.

A more detailed survey was made to show the various loci of different breaks in the chromosome and the data are summarized in Table 10. Only two induced breaks out of the total of 175 were found which have

Table 10. *Distribution of breaks in natural and induced inversions*

	Natural	Induced			Author
1	64*C*–69*D* (Cuernavaca)	Heterochromatic region:		64*C*	K.-A.
2	65*C*–75*C* (Pikes Peak)	,,	,,	65*A*/*B*	K.-A.
3	68*D*–74*B* (Tree Line)	,,	,,	65*C*	H.
4	68*C*–79*A* (Santa Cruz)	,,	,,	65*C*	K.-A.
5	69*C*–76*C* (Oaxaca)	,,	,,	67*C*	K.-A.
6	69*C*–79*A* (Estes Park)	,,	,,	70*B*	K.-A.
7	70*B*–76*B* (Arrowhead)	,,	,,	70*C*	H.
8	70*D*–78*A* (Chiricahua I)	,,	,,	72*A*	K.-A.
9	70*C*–70*B* (Texas)	,,	,,	73*A*/*B*	K.-A.
10	70/71–73/74 (Sequoia I)	,,	,,	75*B*/*C*	H.
11	70/71–77/78 (Klamath)	,,	,,	81*A*	H.
12	71*C*–79*D* (Cowichan)	64*B*–75/76			H.
13	71*C*–81*A* (Ukiah)	65*B*–72*B*			K.-A.
14	72*B*–77*A*/*B* (Hidalgo)	66*B*/*C*–77*B*			H.
15	75*C*–80*A* (Olympic)	67*A*–75*A*			K.-A.
16	76*A*–79*D*/80*A* (Hypothetical)	68*D*–79*D*			K.-A.
17	76*A*–79*D* (Mammoth)	69*B*–77/78			H.
18	76*A*–78*A* (Wawona)	70*A*–72/73			H.
19	77*A*/*B*–81*C* (Sequoia II)	70*A*–74*A*			H.
		71*A*–78*A*			K.-A.
		72*A*–75*B*			H.
		73*C*–75*C*			K.-A.
		75*B*–78*A*			H.
		78*C*–80*C*			H.

H. = Helfer. K.-A. = Koller & Ahmed.

apparently identical loci in section 650. We may conclude therefore that while recurrences of breaks at the same locus must be recognized as possible they are very rare. Only one case is known so far in *D. melanogaster* in which the reported 'reinversion' requires breaks at identical loci (Grüneberg, 1936).

SUMMARY

1. The frequency of X-ray-induced chromosome breaks was determined in 425 larvae of *Drosophila pseudo-obscura*. The cytological analysis of the salivary gland chromosomes has shown that 4500 r. units induced changes in the structure of the chromosomes of 40% of the treated sperm.

2. The frequency of breaks per detectable changed sperm suggests that the same amounts of ionization induce the same number of breaks in *D. pseudo-obscura* as in *D. melanogaster*.

3. Analysis of the distribution of chromosome breaks has shown none of one break. Their absence is due to elimination owing to sister-chromatid reunions at later divisions rather than to non-occurrence.

4. The number of sperms with three, five, and seven breaks is smaller than that with two, four and six breaks respectively. It is suggested that the scarcity of sperms with an uneven number of breaks is due to 'proximity effect' or competition rather than to 'terminal deficiency' effect.

5. The frequency of chromosome breaks in different broods derived from treated sperms of the same parent indicates that in *D. pseudo-obscura* aged over 10 days at the time of treatment there is no differential effect of X-rays on germ cells of different sizes.

6. The frequency of intra-arm rearrangements is higher, and that of interchanges is lower, than expected on the assumptions that (i) the chromosomes are of the same length, (ii) that every chromosome has the same breakability, and (iii) breaks occur at random. It is suggested that the discrepancy between expected and observed frequency is probably due to the difference in viability of intra- and inter-arm rearrangements.

7. The distribution of multiple breaks in recovered sperm indicates that breaks in the chromosomes lead to independent rearrangements rather than to simultaneous exchange among all the altered chromosomes.

8. The frequency of deficiencies and duplications is very small, and is attributed to the lower survival value as compared with that of inversions and interchanges.

9. An analysis of distribution of breaks per unit chromosome length has shown that *XR* has a lower, and the fourth chromosome a higher, frequency than would be expected.

10. A comparison of distribution of natural and X-ray-induced breaks in the third chromosome has shown that the breakage points of induced breaks are distributed at non-random.

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CYTOLOGICAL ANALYSIS OF STRUCTURAL HYBRIDITY IN *RHOEO DISCOLOR* HANCE

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(With Plate 3, containing Figs. 23–29 and Twenty-two Text-figures)

Introduction

The critical study of relationships between chromosomes and nucleoli has opened up important new lines of cytological investigation offering greater possibilities of clear interpretation than was previously anticipated. Evidence obtained from such studies is proving to be very useful in tracing the evolution of the nucleus in various organisms. The very considerable technical difficulties involved in making observations regarding the relationship between chromosomes and nucleoli has been overcome by the application of the nucleolar staining methods (Semmens & Bhaduri, 1939; Bhaduri, 1940*b*). Observations presented in this paper not only substantiate the above claims but also indicate the desirability of a wider application of the new methods. It is well established now that the maximum number of nucleoli in a species represents as a rule the number of satellited chromosomes or secondary constrictions present in the chromosome complement of that species. Observations, previously recorded, which contradict the above generalization require corroboration by more critical methods before they can be accepted. One result of the many observations made with the help of the new technique is a realization that the general contention of an increase in the number of nucleoli in a species can only take place through polyploidy, needs to be carefully reconsidered and modified. In a previous paper (Bhaduri, 1942) it has been suggested that structural changes of chromosomes produced by segmental interchanges between nucleolar and non-nucleolar chromosomes may bring about an increase in the number of nucleoli. The very interesting results obtained from the examination of different species of *Tradescantia* made it apparent that investigations on similar lines in the related genus *Rhoeo* might throw more light on this problem, especially as *Rh. discolor* Hance has been generally accepted as an interchange heterozygote which has undergone a number of non-homologous interchanges during the course of its past nuclear history. The present paper deals mainly with the study of the morphology of the

chromosomes and their relation to nucleoli, and some observations regarding meiosis and sterility of pollen grains have also been recorded.

Material and methods

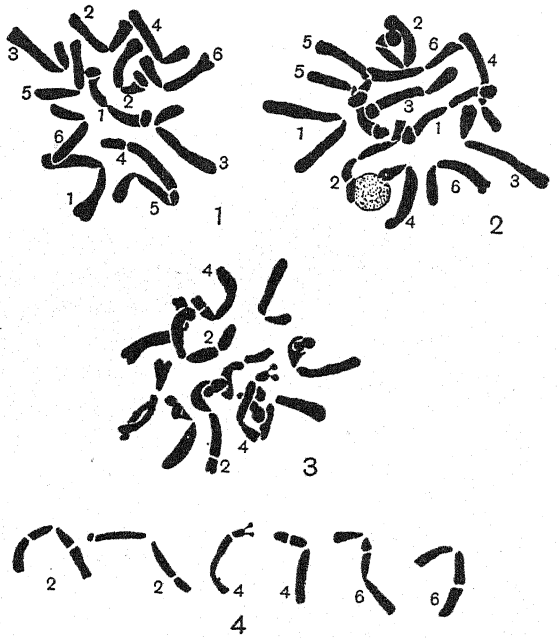
Material for the present study was obtained from plants growing in the University Botanical Garden at Bristol. For a supply of healthy roots young side shoots were cut from adult plants and their bases properly trimmed. They were then put in the propagator for 'striking' and left there for a week, a good crop of healthy roots being produced in this time. As it was found to be very difficult to bring out the secondary constrictions of chromosomes, a number of different fixatives was tried, namely: Benda's with or without acetic acid; La Cour's 2 BE; Lewitsky's 1 : 1 and 6 : 4 chromic formalin mixture; Flemming's medium; Navashin's; and 'Craf'. For showing secondary constrictions best results were obtained from Benda's with low acetic acid, whereas for details of the prophase chromosomes 'Craf' was found most suitable. Both root-tip smears (Bhaduri, 1938, 1940*b*) and paraffin sections 20 μ thick were made. They were stained with Newton's gentian violet iodine method and Feulgen light green method (Semmens & Bhaduri, 1939). For studying meiosis, smears of pollen mother cells and pollen grains were fixed in chrom-uranium combination (Bhaduri & Semmens, 1940) of the following composition: 1·5 g. of sodium di-uranate dissolved in 100 c.c. of 1% chromic acid. They were stained both in gentian violet and in Feulgen light green (Bhaduri, 1940*b*).

The observations and drawings were made using a Zeiss 2 mm. apochromatic objective 1·4 N.A. with achromatic aplanatic condenser 1·3 N.A., homogeneous immersion and compensating eyepiece × 20.

Observations

The study of the morphology of the somatic chromosomes of *Rhoeo* is fraught with the same difficulties as are encountered in the case of *Tradescantia* spp. Though the chromosome number in *Rhoeo* is half ($2n = 12$) that of *Tradescantia virginiana* L., the chromosomes invariably showed foreshortening and twisting at the metaphase plate. It is extremely difficult to make out the correct morphology of the chromosomes, especially the relative positions of the secondary constrictions from metaphase chromosomes alone. It was found that after employing fixatives containing osmic acid, although the primary constrictions of chromosomes became markedly exaggerated the secondary constrictions, however, remained less distinct. For these reasons all the eight secondary

constrictions were never found in a single plate. After examining a large number of metaphase plates as well as after a critical study of the prophase chromosomes the following details were made out. The diploid complement could be roughly distinguished as six pairs; some of the chromosomes, however, showed striking differences from their homologues in their relative lengths and particularly in the position of the secondary constrictions, thus making the whole situation very complicated. This apparent complexity in homology of chromosomes is, however, from



Figs. 1–3. Metaphase plates from root tips of *Rhoeo discolor*. Fig. 4. Chromosomes with secondary constrictions drawn separately. (Benda's with low acetic acid, gentian violet.) ×2800.

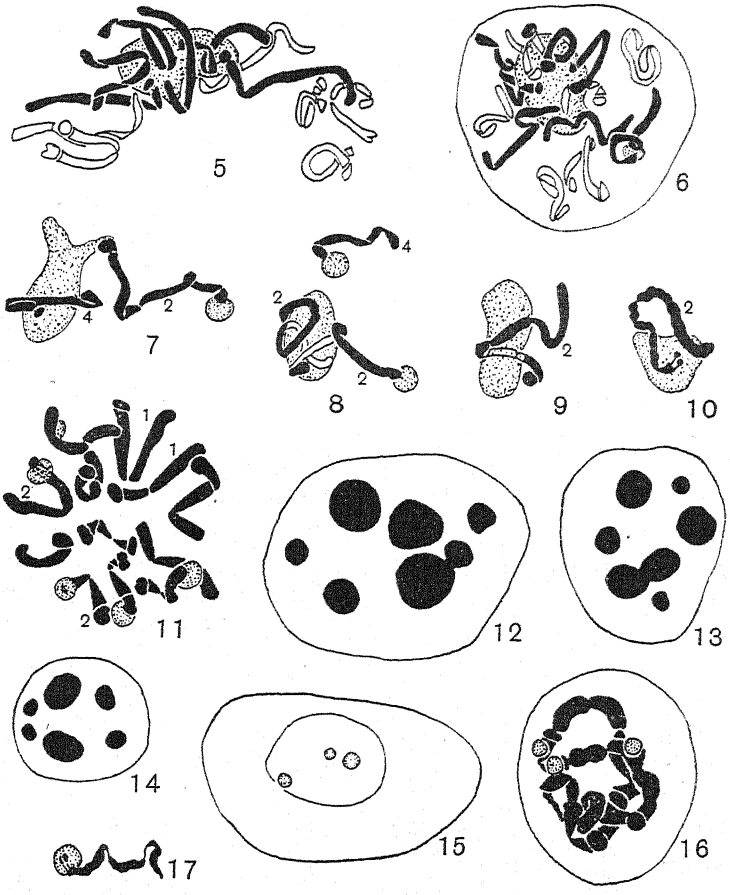
the point of view of the structural hybridity in this species, quite interesting and not very unexpected. A further discussion on this point has been made later in this paper.

The six pairs of chromosomes may be classified as follows: two pairs of chromosomes, the longest in the complement, have median primary constrictions (chromosomes 1, 1 and 2, 2), two pairs of chromosomes with marked submedian primary constrictions (chromosomes 3, 3 and 4, 4); and two other pairs of chromosomes with nearly median, strictly speaking submedian primary constrictions (chromosomes 5, 5 and 6, 6) (Figs. 1–3). Chromosomes 1, 1 have no secondary constrictions and one

is slightly bigger than the other. Both the chromosomes 2, 2 have two secondary constrictions, one in each arm. In one member of the latter pair of chromosomes the secondary constrictions are located more or less in the middle of the arms, whereas in the homologue they are located near the distal ends of the arms (Figs. 23, 24). One of these two distally placed secondary constrictions is located so near the end that it gives the appearance of a typical satellited chromosome (Figs. 4, 10, 24). It seems very likely that this is formed through a non-homologous interchange between a satellited and a non-satellited chromosome. Chromosomes 3, 3 have no secondary constrictions and one is longer than its partner. Chromosomes 4, 4 have both secondary constrictions, one in each of their shorter arms, one of which appears as a typical satellited chromosome (Figs. 3, 4, 25). Both the pairs of chromosomes 5, 5 and 6, 6 are smaller than chromosomes 1, 1 and 2, 2. Chromosomes 5, 5 have no secondary constrictions, but chromosomes 6, 6 each have one secondary constriction; in one it is very near the primary constriction, whereas in the other it is more or less in the middle of the longer arm of the chromosome (Figs. 4, 26, 27). As pointed out before, all the eight secondary constrictions were never found clearly visible in a single metaphase plate.

After very careful study it was concluded that the maximum number of nucleoli in the somatic nuclei of *Rhoeo* is eight, although more than six separate nucleoli in one nucleus were not observed. Figs. 12 and 13 will show at once, however, that the maximum number of nucleoli is certainly higher than six. All the six chromosomes with their total of eight secondary constrictions were found attached to nucleoli during prophase (Figs. 5, 6). From the manner in which these chromosomes were attached to the nucleoli, as well as from the exceptionally lucky spots shown in Figs. 7–9 and 11, it may be safely concluded that all the eight secondary constrictions are nucleolar constrictions, each organizing a separate nucleolus during telophase. Owing to the large number of chromosomes with secondary constrictions placed very close to each other in a relatively small volume of the telophase nucleus the chances of fusion of nucleoli with each other is very great. Further, the two secondary constrictions present in each of the chromosomes 2, 2, especially those two constrictions present in the middle of the arms, are so near to each other that the two nucleoli produced by each of these two constrictions generally get fused with each other. It is for this reason that we find later in the prophase the very characteristic manner of attachment of these chromosomes to nucleoli (Figs. 7–9).

The observations put forward above also explain why eight separate nucleoli were not observed.



Figs. 5–10. Showing the number and manner of attachment of chromosomes to nucleoli during somatic prophase. In Figs. 5 and 6 only those six chromosomes attached to the nucleoli are shaded. Fig. 11. Somatic metaphase plate; note six different chromosomes attached to five different nucleoli (root-tip smear, Lewitsky's 1 : 1). Figs. 12–14. Number and sizes of nucleoli in somatic nuclei. (Figs. 12 and 13, root-tip smear, Lewitsky's 1 : 1; Fig. 14, La Cour's 2 BE, gentian violet.) Fig. 15. Pollen grain nucleus with three nucleoli. Fig. 16. Early diakinesis with three nucleoli. Fig. 17. Satellited chromosome attached to the nucleolus during early diakinesis. (Figs. 15–17. Smear preparations; Lewitsky's 1 : 1; Feulgen light green.) × 1900.

The size relation of the nucleoli in *Rhoeo* could not be made out accurately because the eight separate nucleoli in one nucleus were not observed. The probable size relation seems, however, to be two slightly unequal pairs of small nucleoli and two probably unequal pairs of bigger

nucleoli. The four smaller nucleoli are shown in Fig. 14. It appears also that the two nucleoli formed by the two constrictions of a single chromosome may be unequal in size and non-homologous. According to the segmental interchange hypothesis (Darlington, 1929*a*) this non-homology of nucleoli satisfies fully the theoretical expectations. The fact that the maximum number of six nucleoli actually observed did not show fixed size relationship as theoretically expected (Bhaduri, 1941, 1942) provides, though indirectly, strong evidence in favour of the view that the true maximum number of nucleoli in *Rhoeo* is eight and not six.

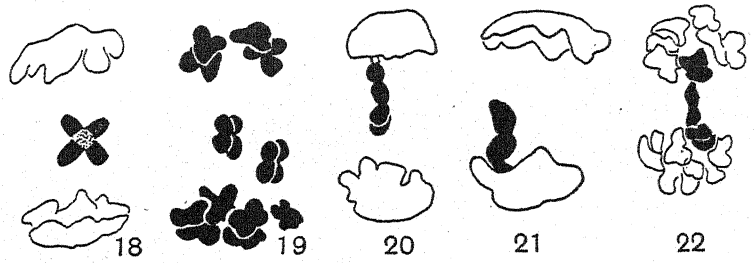
Another characteristic feature noted in *Rhoeo* was the persistence of the nucleoli till late metaphase. Metaphase chromosomes were frequently observed still attached to the persisting nucleoli. Fig. 11 shows clearly how the six nucleolar chromosomes are attached to five distinct nucleoli.

Meiosis in *Rhoeo* has been extensively studied by a number of investigators (Darlington, 1929*b*, 1938; Kato, 1930; Sax, 1931; Sax & Anderson, 1933; Koller, 1932). Koller has shown pairing between homologous segments of non-homologous chromosomes during pachytene. He holds that interstitial chiasmata are formed between these paired segments which by subsequent terminalization form the complete characteristic ring of twelve chromosomes during diakinesis. During the present investigation interstitial chiasmata were not observed though brightly stained (both in Feulgen and gentian violet) peculiar connexions between chromosomes widely separated in the ring have been occasionally observed from pachytene to diakinesis (Fig. 28). These connexions probably indicate true pairing between duplicated homologous segments in non-homologous chromosomes which are widely separated in the ring. The origin of such segments may be explained as due to non-homologous interchanges of one chromosome with two or more other chromosomes. It is interesting to point out here that a fully opened complete ring of twelve chromosomes as found in some ring-forming Oenotheras was seldom observed. It appears that these peculiar connexions probably produce mechanical interference preventing the spreading of the ring, and this may lead to the formation of chains or even to some of the lagging bivalents frequently observed.

Nucleoli in the pollen mother cells were detectable up to diplotene and early diakinesis. More than three separate nucleoli in a pollen mother cell were not observed (Fig. 16). This is due to the fusion of nucleoli during pairing of chromosomes. Pollen grains with three different nucleoli were also clearly observed in a number of cases (Fig. 15). Attachment of chromosomes to nucleoli both by satellites and secondary

constrictions during meiosis has been shown in Figs. 16 and 17. It is to be noted, however, that the nucleoli in the pollen grains are found to be smaller than in the root-tip nuclei.

The separation of the chromosomes during first anaphase is fairly regular, alternate chromosomes in the ring passing to opposite poles. Non-disjunction of chromosomes leading to 7 and 5 distribution has been observed in 18% of the pollen mother cells showing first anaphase. Other complicated types of non-disjunction double non-disjunction in the same or opposite sides, as previously described by Darlington (1929*b*) and Sax & Anderson (1933), have also been observed. When nucleolar chromosomes are involved in this latter type of non-disjunction, pollen grains are produced with a greater or less number of nucleoli than normally expected. Formation of pollen grains with such abnormal



Figs. 18, 19. First anaphase showing lagging chromosomes and the second division split.
Figs. 20, 21. False appearance of bridge formation due to incomplete separation.
Fig. 22. Inversion bridge during first anaphase. × 1900.

numbers of nucleoli have been shown in the case of *Tradescantia* spp. (Bhaduri, 1940*b*).

Lagging chromosomes during first anaphase were frequently observed (Figs. 18, 19). Nearly 45% of the cells showing first anaphase showed the presence of lagging chromosomes. In exceptional cases only two lagging chromosomes in a pollen mother cell were observed. These lagging chromosomes like other univalents of *Rhoeo* showed evidence of second division split already. This second division split was, however, never seen completed at this stage, and the four chromatids were usually seen attached to each other at the middle via less stainable material (Fig. 18). It seems highly probable that a good percentage of lagging bivalents may be produced due to the persistence till first anaphase of one or more chiasmata in a bivalent. These chiasmata are bound to produce mechanical interference during first anaphase, and if two such univalents which are otherwise free separate partially the movement may stretch the chromatids and give the false appearance of an inversion

bridge without an acentric fragment. Darlington (1938) has described some of these peculiar types of chromatid bridges as anomalous types of bridge formation. Besides the irregularities mentioned above true first division bridges not accompanied by acentric fragments were observed in few instances (Figs. 21, 29). Kato (1930) has also reported the presence of such bridges in *Rhoeo*. Darlington (1938), on the other hand, has observed both dicentric bridges with acentric fragments as well as bridges without fragments. He found that the acentric fragments were too small to be seen and were also sometimes rendered invisible due to their location on the surface of the nuclei. According to Darlington (1938) the inversion crossing-over in *Rhoeo* is less than 1 % of cells. Out of hundreds of cells examined he found only three cases of first division and three of second division bridges respectively.

Discussion

Rhoeo is a monotypic genus closely related to *Tradescantia*. It is now grown in many parts of the world for its fleshy, showy leaves. According to Brückner (1930) the native home of this species is Central America and Mexico, though it has been found, probably introduced, in Natal and Jamaica. In spite of the very high percentage of pollen sterility present in this species, above 80 %, it frequently produces viable seeds. According to Anderson & Sax (1936) the progeny grown from seeds breeds true to type. The usual method of propagation practised in gardens is, however, from shoot cuttings. This species has been very critically examined previously by a number of cytologists owing to the fact that it shows the presence of a complete ring of twelve chromosomes during diakinesis. According to the segmental interchange theory described by Darlington (1929*a*) for *Oenothera* spp., *Rhoeo* has been designated as an interchange heterozygote. Darlington (1938), Sax (1931) and Anderson & Sax (1936) explained the ring formation in *Rhoeo* also on segmental interchange basis and have concluded that no two chromosomes in the ring are completely homologous. They found, however, that the order of the chromosomes in the ring was always the same. Koller (1932) has supported the explanation of Darlington and actually observed side by side pairing of homologous segments of non-homologous chromosomes.

Previous observations on the morphology of the somatic chromosomes in *Rhoeo* are very meagre. Darlington (1929*b*) states: 'The attachment constrictions are approximately median except in four chromosomes where the smaller of the two segments is less than half the length of the

longer. Two probably dissimilar chromosomes had small trabants on the shorter arm, and one chromosome was frequently seen to have a second constriction very close to the attachment, separating, as it were, an interstitial trabant (cf. *Tradescantia virginiana* vars. and *Spironema fragrans*). The other subordinate constrictions were less clear and were only occasionally observed.' According to Anderson & Sax (1936), four of the twelve chromosomes have subterminal fibre constriction while the other eight are more or less median.

From the brief account, put forward above, of the previous cytological observations made on this plant it will be noted that the heterozygous nature of this species has been generally accepted. The present observations made on the morphology of chromosomes and nucleoli and their interrelationship provide, however, a new series of more direct and critical evidence proving the structural hybridity of the species. It has been shown, for instance, that although the twelve chromosomes could be paired according to their shapes and sizes, some of the chromosomes show remarkable difference in their morphology, like the position and presence or absence of secondary constrictions, from their respective homologues. The above observation may be taken as conclusive evidence of the presence of non-homologous segments in a chromosome as demanded by the segmental interchange theory. According to this theory (Darlington, 1929*a*) each chromosome in the ring of twelve is homologous at one end with the end of an adjoining chromosome, while at the other end it is homologous to one end of the other adjoining chromosome.

Rhoeo discolor is considered to be a diploid species. Its haploid number ($n=6$) is the same as that of the haploid number of the diploid species of *Tradescantia*. The presence of eight nucleoli corresponding to eight secondary constrictions apparently contradicts the rule of correspondence between the number of nucleoli and the number of genomes present in a species (De Mol, 1928). It also contradicts the view that increase in the number of nucleoli in a species can only be brought about by polyploidy or aneuploidy. McClintock (1934) has shown in maize that an interchange at the point of the nucleolar organizer of the sat.-chromosome with another non-nucleolar chromosome produces two new chromosomes each capable of organizing a nucleolus separately. She has explained further that a homozygous recombination of gametes having such altered chromosomes will produce plants with four nucleoli of different sizes instead of a pair as normally present in the somatic nuclei. The origin of the four nucleoli in the diploid species of *Tradescantia*

has been interpreted on similar lines (Bhaduri, 1942) as suggested by McClintock in the case of some of the maize plants. It has also been pointed out in the same paper that the original hypothesis of De Mol (1928) claiming that diploid species must have two, triploid three and tetraploid four nucleoli needs certain modifications, because many well-known diploid species have four and sometimes more nucleoli in their somatic nuclei. It can be stated now that although true diploid species generally have a pair of homologous nucleoli in their somatic nuclei, increase in the number of nucleoli may occur through certain kinds of structural changes of chromosomes, as well as through increase in the number of chromosomes. In case of *Rhoeo*, where there is no evidence of polyploidy, the presence of eight nucleoli instead of two in the somatic nuclei is to be interpreted on the basis of segmental interchange described above. Cytological and genetical evidence in general corroborates the occurrence of a number of non-homologous interchanges in the past history of the evolution of the nucleus in *Rhoeo*. The origin of eight secondary constrictions has to be looked upon as derived from six interchanges between nucleolar and non-nucleolar chromosomes. Such interchanges may not necessarily have taken place always at the secondary constriction region. It appears that the whole sat.-chromosome behaves as a unit of nucleolar chromosome, although the process of organization of a nucleolus may commence at a particular locus.

Evidence obtained so far leads strongly to the view that size relation of nucleoli in a species is a constant character (Bhaduri, 1938, 1940*a*, 1941). A true diploid species has an identical pair of homologous nucleoli corresponding to the homologous pair of sat.-chromosomes. Change in this size relation of nucleoli may be brought about by structural changes of chromosomes (Bhaduri, in *Scilla* species, unpublished). In the case of *Rhoeo*, although the eight nucleoli could not be observed separately in one nucleus their marked size variation provides further proof of the structural hybridity of this species. Two nucleoli of different sizes attached to two arms of a chromosome not only confirm the above view but also provide additional proof of the presence of non-homologous segments in a chromosome of *Rhoeo*. From the evidence put forward above it will be noted that the study of the morphology of the chromosomes and nucleoli not only helps in following the nuclear evolution in a group of organisms but also throws considerable light on following the nature of changes undergone in the nucleus during the process of evolution.

It has been mentioned before that there is a high percentage of pollen

sterility in *Rhoeo*. Besides the temperature effect (Anderson & Sax, 1936), the main causes of the pollen sterility in *Rhoeo* appear to be due to:

(1) *Deficiency caused by non-disjunction.* Non-disjunction in first anaphase has been found in 20 % of the pollen mother cells. Darlington (1929*b*) concluded that pollen grains with five chromosomes were not viable. It has been shown in the case of *Tradescantia* species (Bhaduri, 1942) that only in polyploid species pollen grains deficient in one or more chromosomes may still survive. In *Rhoeo* deficiency of one chromosome seems to be sufficient to produce complete lethal effect.

(2) *Deficiency caused by irregular disjunction or double non-disjunction on opposite sides:* that is, instead of alternate chromosomes going to opposite poles, two consecutive chromosomes in the ring may go to the same pole. Darlington (1929*b*) has already explained, following the segmental interchange hypothesis, how genetically defective *n* gametes may be produced from a double non-disjunction on opposite sides. Sax & Anderson (1933) have found non-disjunction, including double non-disjunction on the same or opposite sides, in 80 % of the pollen mother cells, which they assume accounts for the sterility of 80 % of the pollen grains.

(3) *Deficiency by lagging chromosomes during meiosis.* It has been mentioned before that 40 % of the pollen mother cells in first anaphase showed the presence of one or rarely two lagging chromosomes. If we assume that all these pollen mother cells showing lagging chromosomes produce pollen grains and knowing that deficiency of one chromosome produces lethal effect, then a high percentage of pollen sterility in *Rhoeo* can at once be accounted for.

SUMMARY

Cytological analysis of *Rhoeo discolor*, with the aid of improved technique, revealed the following important features:

Marked morphological dissimilarity between two members of a chromosome pair could be clearly distinguished although the twelve chromosomes in the somatic nuclei could be definitely classified into six distinct pairs according to the sizes of the chromosomes and the relative positions of the primary and secondary constrictions. Out of the six pairs of chromosomes three pairs had secondary constrictions. One pair (2, 2) shows two secondary constrictions in each chromosome. The chromosome pair 2, 2 showed the presence of at least two morphologically differentiated non-homologous segments. The number and manner of attachment of the chromosomes to nucleoli during prophase shows that

all the eight secondary constrictions are nucleolar. Observations made in the pollen mother cells regarding the chromosome-nucleolus relationship are in agreement with those made in the root-tip cells. Evidence has been put forward that reduplicated homologous segments in non-homologous chromosomes placed widely separate in the ring pair occasionally, which later in the opening out of the ring causes mechanical interference.

The presence of non-homologous segments in a pair of homologous chromosomes has been shown to constitute a definite structural dissimilarity, and this differentiation is shown to arise from previous segmental interchanges. These observations are in agreement with theoretical expectations, as demanded by the segmental interchange theory for interchange heterozygotes.

A high percentage of non-disjunction and of lagging chromosomes during first anaphase satisfactorily accounts for the high percentage of pollen sterility.

The high number of nucleoli in a diploid species like *Rh. discolor* is to be interpreted as due to segmental interchanges between nucleolar and non-nucleolar chromosomes during the course of evolution of this species. The current view that increase in the number of nucleoli in a species can only be brought about by polyploidy or aneuploidy needs to be modified in the light of the present observations.

The present work provides direct cytological evidence for the structural hybridity of *Rh. discolor* and at the same time indicates new possibilities of research on similar lines in other organisms.

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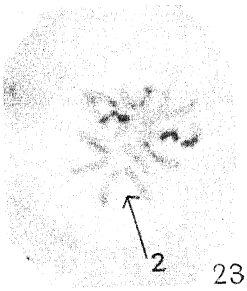

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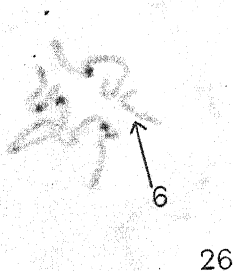

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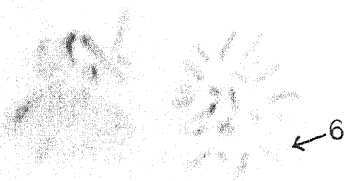

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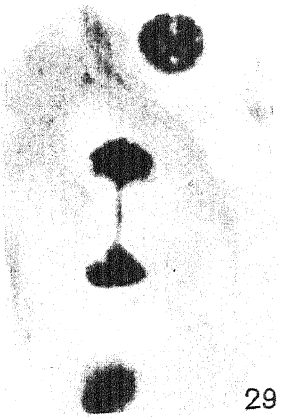

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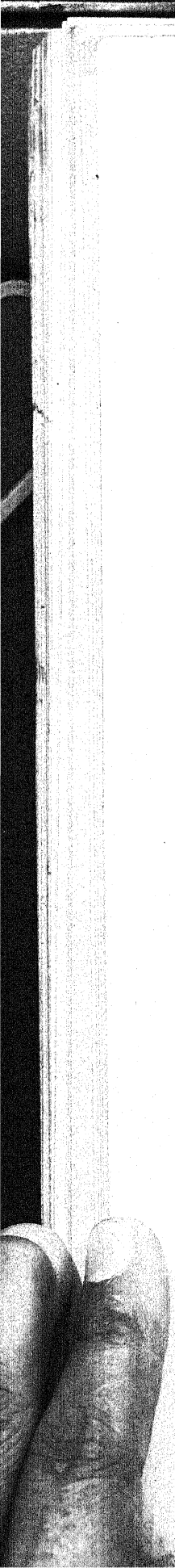

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EXPLANATION OF PLATE 3

Figs. 23–29. Photomicrographs taken with the aid of a Zeiss 'Phoku' apparatus. Initial magnification × 1000.

Figs. 23–27. Somatic metaphase plates of *Rhoeo discolor* showing some of the chromosomes with secondary constrictions. (Excepting Fig. 26, which is taken from a preparation stained with Feulgen, the rest are from preparations fixed in Benda's with low acetic acid and stained with gentian violet.)

Fig. 28. Late pachytene showing attachment of widely separated chromosomes in the ring. (Smear preparation, Lewitsky 1 : 1, Feulgen light green.)

Fig. 29. Inversion bridge during first anaphase.

APPLICATION OF NEW TECHNIQUE TO CYTO-GENETICAL REINVESTIGATION OF THE GENUS *TRADESCANTIA*

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(With Plates 4 and 5, containing Figs. 72–91, and Seventy-one Text-figures)

CONTENTS

1. Introduction

Heitz's theory pertaining to the relationship of sat.-chromosomes to nucleoli has been put to test in a number of widely different plant and animal species. Most of the observations made have substantiated the validity of the theory in general and have opened up many interesting lines of research showing great possibilities. Quite a number of different species and genera of plants have been under investigation for some years in this laboratory in connexion with a study of the general problems associated with chromosome-nucleolus relationship. The results of some of these investigations have already been published (Bhaduri, 1939, 1940, 1941; Gates, 1939). During the course of these observations and through the application of the newly developed nucleolar staining method (Bhaduri, 1940*a*), it was decided that many of the previous conflicting observations might have resulted from inaccurate or incomplete observations due to the serious limitations of the previous techniques. Considerable technical difficulty is experienced for instance with those species which have chromosomes with extremely minute satellites or where the secondary constriction is very near the primary one. Then again, where a nucleus contains a number of varying sizes of chromocentres or heterochromatic bodies, to make an exact count of the maximum number of nucleoli, especially if the nucleus happens to be a small one, is not only extremely difficult with the gentian violet technique but the observations made are also bound to be unreliable. Observations put forward in this paper will not only confirm the correctness of the statement made above but will also indicate the necessity for re-examining a great deal of the previous cytological work.

While making preliminary observations with the pollen grains of some of the *Tradescantia* spp. it was noticed that the number of nucleoli present was not in agreement with the number of satellited chromosomes as given by Darlington (1929*a*). Examination of the related genus *Rhoeo* also revealed the same situation. In order to examine carefully this point and to obtain a better understanding of the chromosome-nucleolus relationship in general, a detailed investigation of different species of *Tradescantia* and the related genera *Rhoeo* and *Zebrina* was undertaken. As *Tradescantia virginiana* and the related species have, according to Darlington (1929*a*), very minute satellites which behave in an exceptional manner it was thought that these would provide very suitable material for illustrating the superiority of the nucleolar staining method. It was also considered that both *Tradescantia* and *Rhoeo* would prove very

suitable for studying the effect of structural hybridity on the general chromosome-nucleolus relationship.

While collecting material for the present study, an isolated plant, much more robust and having larger flowers and leaves than the normal *Tradescantia virginiana* plant was discovered in the Botanical Garden of Bristol University. Preliminary examinations of this plant showed it to be a pentaploid strain. Besides studying its nucleolar condition in relation to polyploidy a comparative study of meiosis in the pentaploid and the tetraploid plants was made. In the present paper only the genus *Tradescantia* has been dealt with. An account of the other genus *Rhoeo* will be presented in a separate paper.

2. MATERIAL AND METHODS

The following species of *Tradescantia* have been examined: (1) *T. crassifolia* Cav.; (2) *T. virginiana* L., U.S.A. (mentioned in the text as Plant no. 1); (3) *T. brevicaulis*; (4) *T. virginiana* L. (pentaploid strain, mentioned in the text as plant no. 2); (5) *T. blossfeldiana*.

The following other subspecies and varieties of *T. virginiana* L. were examined: (i) *alba*; (ii) *caerulea*; (iii) *reflexa*; (iv) *lilacina* and (v) *montana*.

Excepting Plant no. 1 and Plant no. 2 growing in the University Botanic Garden at Bristol, material for cytological observations was collected from plants growing at Kew. Root tips were only collected of Plant no. 1 and Plant no. 2. Only the healthy and vigorously growing roots from freshly struck stem cuttings were taken for fixation. For striking cuttings, young shoots were taken and each was cut just below the second or third internode from the tip. They were then left in the propagator for 2–3 weeks. Root tips were fixed in Lewitsky's chromic formalin combinations 1 : 1 or 6 : 4 and in medium Fleming. Paraffin sections 27–30μ thick were made. They were stained both by the nucleolar staining method and in Newton's gentian violet iodine. Observations were also made from root-tip smears prepared according to the nucleolar staining method (Bhaduri, 1940*a*).

For the study of meiosis and pollen-grain divisions, smear preparations of the pollen mother cells and pollen grains were made following the recently introduced nucleolar staining method (Bhaduri, 1940*a*) as well as the usual gentian violet-iodine method. The following fixatives were mainly used for this purpose: Lewitsky's 1 : 1; Benda's, with or without acetic acid; Navashin's, and the recently introduced chrom-uranium combinations (Bhaduri & Semmens, 1940). It was found that both for meiotic and pollen-grain divisions, the fixation images obtained after

using chrom-uranium combinations are in no way inferior to those obtained by fixatives containing osmic acid. The following combination gives very satisfactory results and is therefore strongly recommended: 1·5 grams of sodium di-uranate dissolved in 100 c.c. of 1 % chromic acid.

3. Observations

I. *Morphology of the chromosomes and satellites*

(i) *Introductory remarks*

The study of the morphology of somatic chromosomes in *Tradescantia* spp. is fraught with serious difficulties. Not only are the chromosomes long and slender, but they show tremendous amounts of foreshortening at the metaphase plate, making it impossible to determine accurately the position of the constrictions and of the very minute satellites present in some of the species (Fig. 1). It is probably for this reason that very

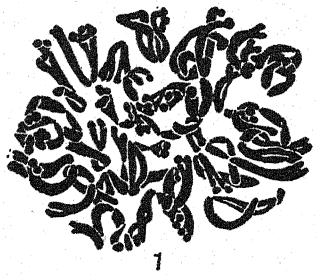


Fig. 1. Metaphase plate from root tip of plant no. 2 ($5n = 30$). Medium Flemming, 27μ, gentian violet. × 1400.

little attention has been previously paid to these points, although some of the species and varieties of this genus have been subjected to critical cytological observations by many eminent cytologists. Darlington (1929*a*) has published the only systematic account of the morphology of the chromosomes in a number of different species of *Tradescantia*. While referring to the satellites of *T. virginiana* he states: '...one or two can frequently be seen with trabant. This trabant is so small that its behaviour is probably exceptional.' During the present investigation with the aid of the improved technique it was soon realized that much more critical observations could be made.

The study of the comparative morphology of the chromosomes in most of the species of *Tradescantia* is further complicated by the fact that the chromosomes do not show marked morphological dissimilarity. To quote Darlington (1929*a*): 'Most of the forms studied have 24 chromosomes varying slightly in size with approximately median primary con-

strictions.' According to Anderson & Sax (1936) 'The chromosomes of the typical Tradescantias have median or submedian primary constrictions. There is no consistent difference in the chromosome size of different diploid species or within the tetraploid forms, but the tetraploids in general have somewhat shorter chromosomes than have the diploids.'

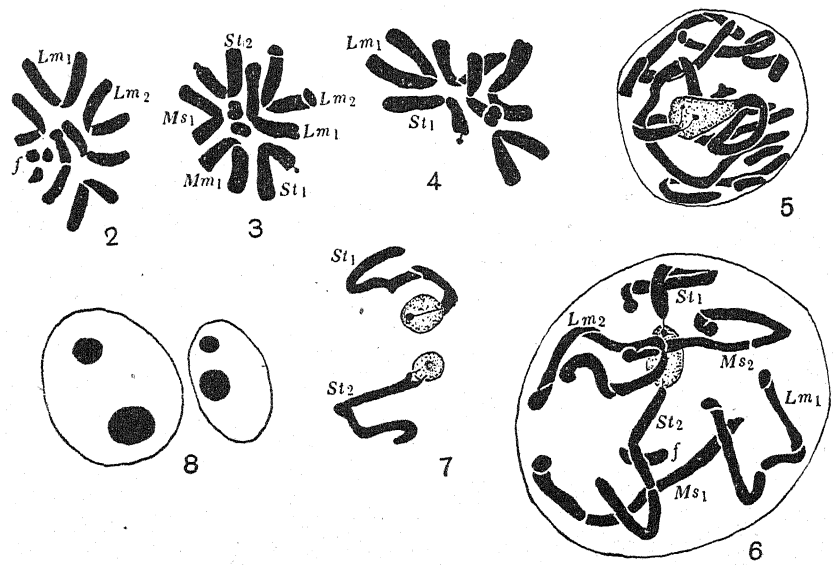
The microspores of *Tradescantia* spp., on the other hand, are very suitable materials for a general study of the chromosomes. Due to relatively poor fixation of the metaphase chromosomes the very small satellites present in most of the species are very difficult to detect. By following the nucleolar staining method, it was found that during prophase the extremely minute satellites can be picked out as bright magenta-coloured bodies or threads against the green background of the nucleolus. It may be suggested here, therefore, that the difficulties involved in finding out very minute satellites, like those of *Tradescantia* spp., may be overcome by using this new staining method (Bhaduri, 1940*a*) and searching for the satellites in the prophase chromosomes instead of in metaphase ones as usually practised. The observations on the morphology of the chromosomes as presented below have chiefly been made from the first division of the microspores.

(ii) *Observations in individual species*

Tradescantia crassifolia Cav. ($n=6+2f$). After careful observations and comparison of a large number of plates, it was found that the haploid chromosome complement of this species could be grouped as follows: Two long, but slightly unequal, chromosomes with median primary constrictions (Lm_1, Lm_2). Two shorter chromosomes, one with median and the other with submedian primary constrictions (Ms_1, Mm_1), and two others which are satellited, one with slightly submedian and the other with median primary constrictions (St_1, St_2) (Figs. 2–4, 74–75). The satellite of the former is represented by a knob and a filament, while in the latter it is represented only by a minute filament almost at the limits of visibility (Figs. 3–7, 75). The three small fragments (*f*, Fig. 2) really represent two small chromosomes with almost terminal primary constrictions. The range of chromosome lengths in this species was found to be between 5 and 8 μ, the size of the fragments being 1·5 μ. According to Darlington (1929*a*) the chromosomes of this species resemble those of *Rhoeo*. From his figures it appears that he did not find any satellited chromosomes.

Tradescantia virginiana L., U.S.A. ($4n=24$). After examining a very large number of plates the twelve chromosomes of the microspores were

grouped as follows. Two slightly unequal pairs of chromosomes, the longest of the complement, with median primary constrictions (Lm_1 and Lm_2). Two unequal pairs of chromosomes with submedian primary constrictions (Msm_1 and Msm_2). Two other pairs of chromosomes with nearly median primary constrictions (Mm_1 and Mm_2) (Figs. 9, 78). There are three chromosomes with satellites and one with a secondary constriction very near the primary one. The satellites of two chromosomes are quite



Figs. 2–4. Metaphase plates from pollen grain divisions. Figs. 5, 6. Prophase, showing the number and manner of attachment of sat.-chromosomes to the fused nucleolus. Fig. 7. Sat.-chromosomes attached to two different nucleoli in a nucleus drawn separately. Fig. 8. Vegetative and generative nuclei with two nucleoli in each. ×1860.

conspicuous, whereas in the third the satellite is only represented by a minute thread (Figs. 21–23). Darlington (1929*a*) found only three to four chromosomes with satellites in the somatic complement. He has occasionally observed the presence of a secondary constriction near the primary one. The range of chromosome length in this species was found to be between 8 and 11 μ.

Tradescantia brevicaulis ($3n=18$). Due to high percentage of non-disjunction and other irregularities various combinations of chromosomes were noted. As shown in Table 4 the chromosome number was found to vary between six and eleven. Pollen grains with nine chromosomes even showed dissimilarity in their idiogram (Figs. 10–13, 76, 77). Typical cases showed three long chromosomes with median constrictions differing

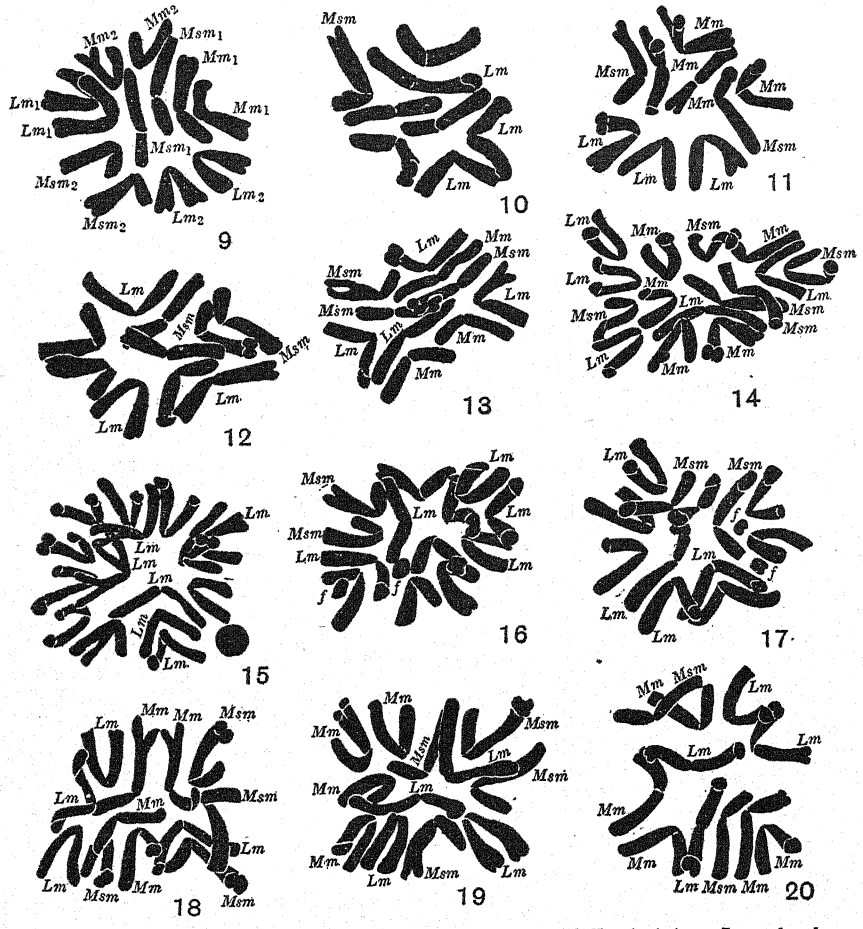
slightly in length or three chromosomes with submedian constrictions. The other type of chromosomes with nearly median primary constriction, as found in the tetraploid species, is also present in the complement. The variation in the idiogram due to non-disjunction has been shown in Figs. 10–13. As root-tip cells in this case were not examined it is difficult to state exactly the number and morphology of the satellited chromosomes. From microspore analysis, however, it was found that both types of satellited chromosomes as well as a chromosome with secondary constriction, as observed in the tetraploid species, are also present in this triploid species (Fig. 24).

Tradescantia virginiana L.; plant no. 2 ($5n=30$). Due to non-disjunction pollen grains were found to have chromosome numbers ranging from thirteen to seventeen (Table 4), thus making it extremely difficult to find out the idiogram of this species. In the root-tip cells also, although thirty chromosomes could be counted in some plates, idiogram studies from such plates were impossible due to foreshortening and twisting of the chromosomes (Fig. 1). The comparative morphology of the chromosomes clearly revealed, however, the autopentaploid condition of this species. For instance, in some pollen grains five or six long chromosomes with median constrictions or five or six chromosomes with submedian constrictions have been frequently observed (Figs. 14, 15). Similarly four satellited chromosomes, besides the chromosome with a secondary constriction near the primary one, have been observed. The size difference between the longest and the shortest chromosome was found to be 11–8 μ.

Tradescantia virginiana L., *montana* (pentasomic; $4n+1=25$). Darlington (1929*a*) first showed that this form is a pentasomic with twenty-five chromosomes and four fragments in the root-tip cells. During the present investigation it was found that pollen grains have ten to fourteen chromosomes with 0–4 fragments in each grain. With regard to chromosome complement, size of the chromosomes or the general morphology of the pollen grains, there is hardly any noticeable difference from that of the tetraploid species (Figs. 16, 17). That the extra chromosome is neither satellited nor has a secondary constriction is shown by the fact that there is no increase in nucleolar number nor in the number of chromosomes attached to the nucleoli as compared with the tetraploid species.

Tradescantia virginiana L., *caerulea*, *alba*, *lilacina* and *reflexa* ($4n=24$). Excepting *caerulea* all the above forms showed chromosome complement identical to *T. virginiana* L., U.S.A. (Figs. 18–20). According to Dar-

lington (1929*a*) var. *caerulea* (double) has twenty-five chromosomes and one fragment in the root-tip cells. During the present investigation, pollen grains of this form showed twelve chromosomes and 0–4 fragments,

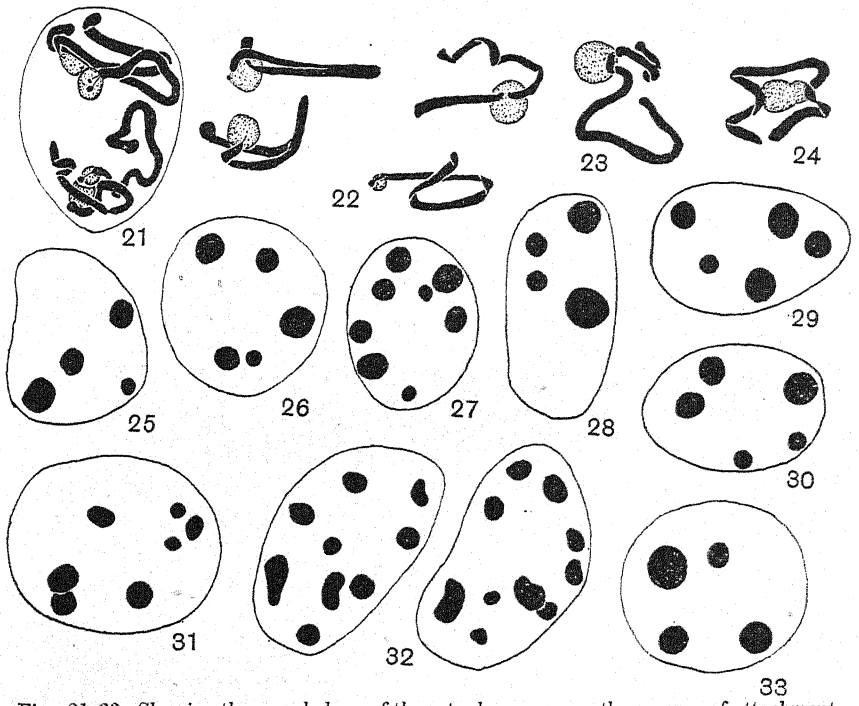


Figs. 9–20. Metaphase plates from pollen-grain divisions of *T. virginiana* L. and other related forms showing the comparative morphology of the chromosomes. Fig. 9. Plant no. 1. Figs. 10–13. *T. brevicaulis* (triploid). Figs. 14, 15. Plant no. 2 (pentaploid). Figs. 16, 17. *montana* (pentasomic). Fig. 18. *lilacina*. Fig. 19. *reflexa*. Fig. 20. *alba*. ×1400.

the correct number being $12+2f$ (Fig. 81). The number and morphology of the sat.-chromosomes as well as the chromosome with secondary constriction appear to be the same as that described for *T. virginiana* L., U.S.A. The above observations clearly show, therefore, that these well-

known varieties and subspecies cannot be correlated with any distinguishable morphological change in the chromosome complement.

Tradescantia blossfeldiana ($n=35$). This Mexican species has thirty-five chromosomes in the pollen grains. The chromosomes are much smaller

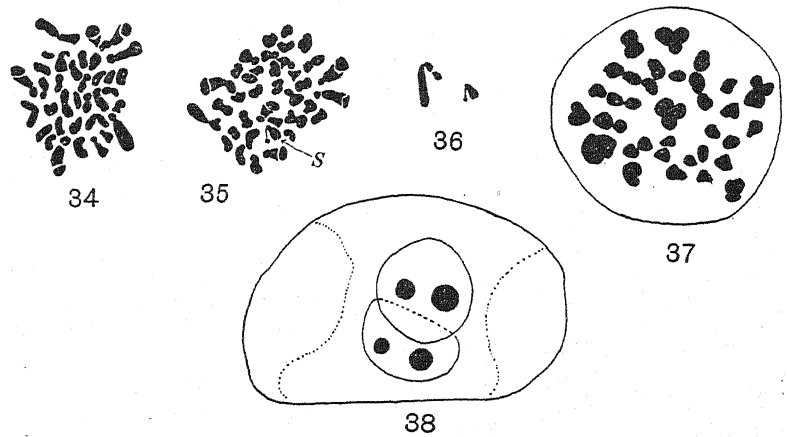


Figs. 21–33. Showing the morphology of the sat.-chromosomes, the manner of attachment of sat.-chromosomes to nucleoli, and the number and size relation of nucleoli in *T. virginiana* L. and the related forms. Figs. 27 and 32 are drawn from root-tip nuclei, the rest are from the first microspore division. Fig. 21. Plant no. 1: note the four chromosomes attached to four different nucleoli. Fig. 22. The four nucleolar chromosomes of plant no. 1 each attached to its respective nucleolus drawn separately. Figs. 23, 24. The chromosome with a secondary constriction near the primary one in *reflexa* and *T. brevicaulis* respectively. Figs. 25–26. Plant no. 1. Fig. 25. Microspore with four nucleoli of three different sizes. Fig. 26. Microspore with five nucleoli due to non-disjunction. Fig. 27. Root-tip nucleus with four pairs of nucleoli. Figs. 28–30. *T. brevicaulis* showing different number and size relation of nucleoli due to non-disjunction. Figs. 31, 32. Plant no. 2. Fig. 31. Microspore with seven nucleoli due to non-disjunction. Fig. 32. Root-tip nuclei, one with ten and the other with nine nucleoli. Note the size relation of nucleoli. Fig. 33. *reflexa*; microspore with four nucleoli of three different sizes. × 1400.

than those of the '*virginiana* group', the range between the longest and the shortest chromosomes being 4·5–1 μ. Eight long chromosomes could be distinguished in the complement (Figs. 34, 35). One small chromosome with a satellite and a long chromosome with a secondary constriction in

the shorter arm were observed (Fig. 36). These two chromosomes were also found attached to the nucleolus during prophase.

The range of variation in the lengths of chromosomes in different species of *Tradescantia* has been presented in Table 3. The same fixative was employed in each case. All the measurements were taken from camera lucida drawings. Darlington (1929*a*) has suggested that a good deal of variation in chromosome length is due to the action of the fixative. No explanation is given for his extraordinary view, that the length of metaphase chromosomes during first pollen-grain divisions should be the same as that found in the root-tip nuclei.



Figs. 34–38. *T. blossfeldiana.* Figs. 34, 35. Metaphase, first division in the microspore showing the number of chromosomes ($n=35$) and their size differences. Fig. 36. Sat.-chromosomes drawn separately. Fig. 37. First metaphase of the pollen mother cell. Note the number and size differences of the chromosomes. Fig. 38. Vegetative and generative nuclei in the microspore showing the size difference of the nucleoli. × 1860.

II. *The relationship of sat.-chromosomes to nucleoli*

(i) *Correlation between maximum number of nucleoli and the number of sat.-chromosomes or secondary constrictions*

A close relationship between the number of satellites and secondary constrictions on the one hand and the maximum number of nucleoli on the other has been observed in all the species and varieties of *Tradescantia* studied. The observations are summarized in Table 1.

From this table it will be seen that the variation in the number of sat.-chromosomes in the pollen grains, especially of the triploid and pentaploid forms, as well as the maximum number of nucleoli, varies. This apparent anomaly I consider to be due to a high percentage of non-disjunction, which has been observed at first anaphase. Diploid species

Table 1

Name of species, subspecies or varieties of *Tradescantia*	Chromosome no.	No. of sat.-chromosomes in somatic and pollen-grain complement	No. of chromosomes with secondary constrictions in the pollen grain	Maximum no. of nucleoli in root-tip nuclei	Maximum no. of nucleoli in pollen grains	Size relation of nucleoli in pollen grains	Size relation of nucleoli in root-tip nuclei	No. of chromosomes attached to nucleoli in the pollen grains
T. crassifolia Cav.	$n=6+2f$	—*	None	—	2	1 big, 1 small	—	2
T. virginiana L., U.S.A.	$4n=24$	6 3	1	8	4–6	1 big, 1 small and 2 intermediates	2 big, 2 small and 4 intermediates	4–6
T. brevicaulis	$3n=18$	— 3–5	1	—	3–5	3 types in varying numbers	—	3–5
T. virginiana L., U.S.A., plant no. 2	$5n=30$	8 4–6	2–1	10	5–7	,,	2 big, 2 small and 6 intermediates	—
T. virginiana L., *montana*	$4n+1=25$	— 3	1	—	4–5	,,	—	4–5
T. virginiana L., *caerulea*	$4n=24+4f$	— 3	1	—	4–5	1 big, 1 small and 2 intermediates	—	4
T. blossfeldiana	$n=35$	— 2	None	—	2	1 big, 1 small	—	2

* — = not examined.

related to *T. virginiana* have not been examined during the present investigation. It is unfortunate that the satellite and nucleolar condition observed in the tetraploid forms of *T. virginiana* cannot therefore be compared to the related diploid species but has to be conjectured from the conditions observed in the other diploid species examined, namely, *T. crassifolia*. The presence of the sat.-chromosomes and one chromosome with a secondary constriction in the complement of the pollen grains of *T. virginiana* at once suggests that the tetraploid condition does not represent a simple duplication of a set of chromosomes. Similarly, the presence of a chromosome with secondary constriction besides two other types of sat.-chromosomes in the triploid species *T. brevicaulis*, introduces further complications. An explanation of the apparently anomalous conditions of sat.-chromosomes in the triploid and tetraploid forms will be presented later in this paper.

(ii) *Size relation of nucleoli*

The size relation of the nucleoli in *Tradescantia* spp. is well marked and consistent. The present observation, in addition to those previously recorded (Bhaduri, 1939, 1940, 1941), shows clearly that the size relation of nucleoli in a species, like the length of chromosomes in a complement, is a fixed character. The two intermediate-sized nucleoli observed in the tetraploid forms often show slight differences in their sizes, and it is very probable that they do not represent a homologous pair but represent two different types. In a solitary exceptional case, however, in the tetraploid species, instead of eight nucleoli an additional small nucleolus was observed. Slight fluctuations in the sizes of the nucleoli also were noted in the pentaploid species. That the size relation between nucleoli in a species is a fixed character is also clearly shown in the vegetative and generative nuclei of the pollen grains. It was found for instance that though the nucleoli in the generative nuclei are as a rule proportionally smaller than those of the vegetative nuclei, the size relations between the nucleoli in each case were constant (cf. Table 2).

(iii) *Attachment of chromosomes to nucleoli during prophase*

From Table 1 it will be noticed that the number of chromosomes found attached to nucleoli at prophase corresponds exactly not only to the maximum number of nucleoli but also to the number of sat.-chromosomes and chromosomes with secondary constrictions present in that particular species. The number of attachments of chromosomes to the nucleoli can therefore be used as a reliable guide to the exact number of

nucleolar chromosomes or the maximum number of nucleoli present in a species.

(iv) *Correlation between nucleolar size and satellite size*

It has already been mentioned that there are two types of sat.-chromosomes found in different species of *Tradescantia*, one with a conspicuous head and a filament and the other with a minute filament only. In the case of *T. crassifolia*, it will be noticed from Fig. 7 that the bigger satellite is always associated with the bigger nucleolus and the smaller one with the smaller nucleolus. In the case of *T. virginiana*, however, it was found that the smallest nucleolus is always associated with the bigger satellite, whereas the chromosome with the satellite represented by a minute thread is only attached to one of the intermediate-sized nucleoli,

Table 2. *Showing the size relationship of nuclei and nucleoli in the pollen grains of* Tradescantia crassifolia

		μ
Diameter of the nuclei:		
Vegetative	—	12·5
Generative	—	9·0
Diameter of the nucleoli:		
Vegetative: Big	—	3·2
Small	—	1·9
Generative: Big	—	2·1
Small	—	1·25

the chromosome with secondary constriction being attached to the biggest nucleolus (Figs. 21, 22). This observation alone proves, therefore, that no generalization as to a correlation between satellite size and nucleolus size could be made. In the case of *Oenothera*, it was shown also (Bhaduri, 1940) that there was no correlation between the length of the satellite filament and the size of the nucleolus. It seems clear, therefore, that the size of a nucleolus is a function of the entire sat.-chromosome. It is interesting to note, however, that from observations made so far, dimorphism of satellites has always been found associated with dimorphism of nucleoli. From this point of view it will be very interesting to note the conditions of heteromorphic races of *Galtonia caudicans* shown by Navashin (1927). During the present observation, root tips of a number of bulbs of *G. candicans* were examined, but in each case a homomorphic pair of satellites was observed. A pair of nucleoli, almost of the same size, was also observed in each case. It is interesting to point out that material examined by Smith (1933) also showed a single homomorphic pair of satellites.

III. *Relationship between sizes of the pollen grains, pollen mother cells, and chromosomes to polyploidy and aneuploidy*

The sizes of the pollen grains, pollen mother cells, and chromosomes and the nuclear volume, in relation to polyploidy are presented in Table 3. It will be noticed that doubling the chromosome set has a proportional effect on the cell and nuclear volume of the pollen grains, although no apparent effect is produced on the size of the pollen grains themselves. The volume of the pollen grains was calculated from the formula for an ellipse $\frac{4}{3}\pi \frac{a^5}{b^2}$ and the nuclei taken as spheres, $\frac{4}{3}\pi r^3$ respectively. It will be noticed further from Table 3 that the presence of

Table 3

Name of the species	Diameter of P.M.C. in microns during diakinesis	Average length of pollen grains in microns	Volume of the pollen grains in cubic microns divided by 1000	Average nuclear volume during prophase of pollen grains in cubic microns divided by 1000	Range of chromosome length in microns in pollen grain	Haploid no. of chromosomes in each species
T. crassifolia	—	34	41·3	5·20	5–8	$n=6+2f$
T. virginiana L., plant no. 1	28	42	101·2	8·13	8–11	$n=12$
T. brevicaulis	—	38	72·0	6·54	8–11	$n=9$
T. virginiana, plant no. 2	30	45	127·7	10·42	8–11	$n=15$
T. virginiana, montana	—	42	101·2	8·13	8–11	$n=12+1$
T. virginiana, caerulea	—	42	101·2	8·13	8–11	$n=12$
T. virginiana, lilacina	—	42	101·2	8·13	8–11	$n=12$
T. blossfeldiana	23	24	13·1	1·43	1–4·5	$n=35$

an extra chromosome in the set of the pentasomic form *montana* or the two fragments in the case of the variety *caerulea* have not induced any marked change in the size or volume of the pollen grains or nuclei. The proportionate increase in the size of the pollen grains followed by polyploidy within a species is well known. The same relation, however, does not always hold good in the case of related species. Kihara & Ono (1926), for instance, found that the size of the pollen grains in *Rumex hydrolapathum* with chromosome number $2n=200$ is not greater than in the related diploid and tetraploid species with chromosome numbers $2n=20$ and 40 respectively. From the above table it will be noticed also that *Tradescantia blossfeldiana*, with thirty-five chromosomes in the pollen grain nuclei, has smaller pollen grains and nuclei than the diploid species, *T. crassifolia*, with $n=6+2f$ chromosomes. It seems, therefore, that in the genus *Tradescantia*, *T. blossfeldiana* represents quite a different line of evolution from the *virginiana* group as is also indicated by the difference in the size and morphology of their chromosomes

(Figs. 34–38). It will be noticed from Table 3 that although the chromosomes in the diploid species are smaller than those of the triploid or tetraploid, no marked differences in size are present between the triploid, tetraploid and the pentaploid forms of *T. virginiana*. Both Darlington (1929*a*) and Anderson & Sax (1936) found, however, that the chromosomes in the tetraploid form are smaller than the diploids. The different forms of *T. virginiana* including the pentasomic form *montana* and the form *caerulea* with two extra fragments did not show any marked difference in the sizes of the chromosomes. The difference in the sizes of the chromosomes in each complement was also found fairly constant in all the above related tetraploid forms. The size relation of the chromosomes in *T. blossfeldiana* is, however, very marked (Figs. 34, 35, 37, 79).

Table 4

Chromosome nos. in pollen grains of triploid and pentaploid plants	6	7	8	9	10	11	12	13	14	15	16	17
Frequency (no. of counts made)	1	8	21	25	27	9	3	29	65	70	39	5

It will be noticed from Table 4 that the triploid species *T. brevicaulis* and the pentaploid form plant no. 2 have pollen grains with chromosome numbers varying from six to eleven and twelve to seventeen respectively. The frequency of their occurrence in each plant has been presented also in Table 4. The percentage of abortive pollen grains in the triploid plant was found to be as high as 55 %, whereas in the pentaploid it was approximately 20 %. This difference in the growth of the pollen grains is also reflected in the morphology of the two plants, the triploid being a dwarf plant with reduced flowers and narrow leaves, whereas the pentaploid is a much more robust plant with longer leaves and flowers (Fig. 72). The triploid plant *brevicaulis*, for various reasons, is considered to be an allotriploid, which arose from a cross between different tetraploid and diploid parents. The pentaploid, on the other hand, is considered to be an autopentaploid plant of *T. virginiana* U.S.A.

The wide range of variation in the chromosome number of the pollen grains in these two plants offered a good opportunity to study the relation of pollen-grain size to the chromosome number. The data obtained during the present study have been presented in Table 5. The size differences in pollen grains between the triploid, tetraploid and pentaploid plants of *T. virginiana* are not so well marked as expected. Under the microscope this difference is hardly detectable. The volume relation between the pollen grains of the three is, however, quite significant (Table 3). According to Sax (1937), 'The young microspores of the tri-

ploid are about twice as large as those of the diploid at the corresponding stage, but the variability is no greater. These microspores also double their size by the time the nuclei divide, but at this stage the variability is greatly increased.' The above statement made by Sax is, however, not in agreement with the data he has presented as evidence (Sax, 1937, p. 528). His measurements, however, are in agreement with that made during the present study as will be seen in Table 3.

It must be pointed out here, as a caution, that a considerable amount of variation in measurements of the pollen grains in fixed smear preparations was found to be due to unequal stretching of the pollen grains brought about by the action of the fixatives. The first action of an aqueous fixative on the pollen grains seems to be stretching of the wall due to imbibition of water. This reaction is variable with different fixatives employed. The measurements obtained from using two fixatives, Lewitsky's 6 : 4 and chromic-uranic combination (Bhaduri & Semmens, 1940), have been presented in Table 5. With uranic-chromic combination the grains appeared to be fully extended, whereas with Lewitsky's the extension was not uniform, showing effect of shrinkage here and there. Fixation of the chromosomes in both cases was, however, almost identical. It is, therefore, most important to get measurements from pollen grains which are uniformly distended.

Darlington (1929*a*) has previously studied the relation of pollen grain size to chromosome number in the triploid species *T. brevicaulis*. He holds the view that the growth of the pollen grains depends on the balance between 'chromatin elements'. He presumably considers this plant as an autotriploid where some of the chromosomes in the complement are only duplicated. He considers that the scale of unbalance of the pollen grains varying in number from six to twelve will be somewhat in the degree of 0/15, 5/15, 8/15, 9/15, 5/15, 0/15. He found also that pollen grains with eight chromosomes were as a rule smaller than those with seven, which he considers can be interpreted on the basis of the degree of unbalance, as shown in the above scheme. He concludes 'the evidence from *brevicaulis* shows a sharp differentiation between the members of the chromosome complement in *Tradescantia* but that the variation in size of the pollen grains with numbers from 8 to 10 shows that the degree of differentiation of the several chromosome types is not equivalent'. Sax (1937) has also studied the relation of pollen-grain size to chromosome number in a triploid plant of *T. bracteata*. This plant has been considered by Sax as an autotriploid plant. His conclusions on this point may be quoted here: 'The variability in size of microspores is greatly increased

by differences in chromosome balance, and the variability decreases as the chromosome number approaches a normal balance of 6 or 12. Evidently different chromosome combinations, even where the number is the same, have different effects on cell growth. Microspores with eight chromosomes may be smaller than the average size of haploid microspores. It is clear that both chromosome number and chromosome balance have a direct and immediate effect on the growth of the microspore.'

Table 5. *Relation of chromosome numbers to pollen-grain sizes in polyploid forms of* Tradescantia virginiana

Chromosome no.		Lengths of pollen grains in microns, and below number of cases observed											
		34	36	37	38	40	42	44	46	48	50	52	54
T. brevicaulis	6	1	.	.	.	.	.	.	.	.	.	.	.
	7	1	1	2	1	.	.	.	.	.	.	.	.
	8	5	1	.	1	1	1	.	.	.	.	.	.
	9	1	3	2	3	4	4	.	.	.	.	.	.
	10	.	1	.	2	1	3	.	.	.	.	.	.
	11	.	.	.	1	2	6	.	.	.	.	.	.
	12	.	.	.	.	.	.	.	.	.	.	.	.
Plant no. 1	12	.	.	.	3	2	7	2	1	.	.	.	.
Plant no. 2	12	.	.	.	.	0, 1*	0, 1	0, 1	.	.	.	.	.
	13	.	.	.	0, 1	3, 4	5, 1	6, 4	.	0, 2	.	.	.
	14	.	.	.	1, 0	7, 0	10, 6	2, 7	0, 8	0, 2	.	.	.
	15	.	.	.	3, 0	6, 2	17, 5	5, 10	1, 10	.	.	1, 0	1, 0
	16	.	.	.	.	3, 0	4, 0	3, 6	1, 5	0, 1	0, 1	.	.
	17	.	.	.	.	.	2, 0	.	.	0, 1	.	1, 0	.

* The first and second figures here represent counts made from smears fixed in Lewitsky's 6 : 4 and chrome-uranium fixation respectively.

It will be noted from Table 5 that pollen grains with the same chromosome number show a marked variation in their sizes. There is also a direct relation between chromosome number and pollen-grain size in both the triploid and pentaploid plants studied. Contrary to Darlington's presumption this triploid plant *T. brevicaulis*, as already mentioned, is considered to have been derived from a cross between different diploid and tetraploid parents. Therefore, only pollen grains having the full complement representing both the parents are capable of normal growth and development. On the other hand, pollen grains with less than nine chromosomes, up to seven, being deficient of one or two chromosomes from the full (balanced) complement, are, as a rule, incapable of full growth and development and are mostly smaller than the average size of healthy pollen grains. Pollen grains with less than seven chromosomes, which appears to be a limiting number, fail to mature. The marked irregularity and abnormal behaviour observed in these grains during

divisional stages corroborates this view. During the present study only two pollen grains with six chromosomes were observed. In both instances the chromosomes so observed were found irregularly scattered without showing any sign of polarity. In one the chromosomes were found to be precociously divided; the chromatids were, however, still undivided at the region of primary constriction (Fig. 59). Pollen grains with ten or eleven chromosomes, i.e. with one or two more chromosomes than the haploid complement, develop normally but show, on average, an increase in size. Pollen grains with eleven chromosomes on the average attain the average size of the tetraploid parent. Whether there is a limiting number on this side also, checking the normal growth of the pollen, is not known, because pollen grains with twelve chromosomes have not been observed. It appears from the tables given by Darlington (1929*a*) and Darlington & Upcott (1941) that they also have not seen pollen grains with twelve chromosomes. In the case of pollen grains with nine chromosomes, more than 60 % show normal growth and development. The variation in sizes observed in other grains may be explained as due to irregular disjunction of some of the homologous chromosomes during first meiotic division, making these grains deficient in one or two chromosomes while duplicating one or two other chromosomes. Upcott (1937) has also pointed out that in the tetraploid and pentaploid species of *Tulipa*, the entire diploid set of chromosomes being present in many cases, the pollen grains are especially protected against deficiency.

Also, in the case of the pentaploid plant, a considerable amount of variation in size of the pollen grains was noted, and this variation can be explained on the same principle as stated for the triploid plant. This pentaploid plant is an autopentaploid, some of the chromosomes of the tetraploid complement being duplicated. This is shown by the marked drop in the percentage of sterility of the pollen grains. Pollen grains with chromosome numbers ranging from thirteen to sixteen being provided with the full tetraploid complement grow normally but show almost the same degree of variation in their sizes, comparable to the variation of sizes observed in the pollen grains of *T. brevicaulis* and in the tetraploid *Tradescantia virginiana* plant (Table 5).

It is interesting to note, however, that the average size of the grains with twelve to fourteen chromosomes is lower than the average size of grains with sixteen or seventeen chromosomes, showing a direct relation, though within a narrow limit, between the size of the grain and the chromosome number. Increase in chromosome number higher than sixteen seems to introduce extreme conditions and the pollen grains show abnormal growth. This is further verified by the presence of con-

siderably higher numbers of grains with thirteen chromosomes than with sixteen.

From the observation put forward above it will be noted that, generally speaking, the data obtained for the triploid species *T. brevicaulis* are in agreement with those obtained by Sax (1937) in the triploid *T. bractiata* and Darlington in *T. brevicaulis*. Conclusions based upon measurements obtained from a few pollen grains only are not likely to be significant, and the coincidence of pollen grains with eight chromosomes being smaller than those with seven as reported by Darlington (1929*a*) is probably due to only a small number of pollen grains being examined. Although the variation in pollen-grain size may be explained as due generally to chromosome unbalance, the important aspect in the interpretation of this relationship between chromosome number and pollen-grain size presented above seems to have been primarily overlooked. The data presented for the pentaploid plant definitely show that the degree of variation in size does not follow any scale of unbalance of chromosome number. Further, it appears that the nature of unbalance in the grains with seven or eight chromosomes is not comparable to that with ten or eleven chromosomes. It is shown also that increase in chromosome number above the balanced condition, that is, grains above the full complement, tends to increase the size of the grains.

IV. *Meiosis*

(i) *Pairing of chromosomes in tetraploid and pentaploid* T. virginiana *L.*

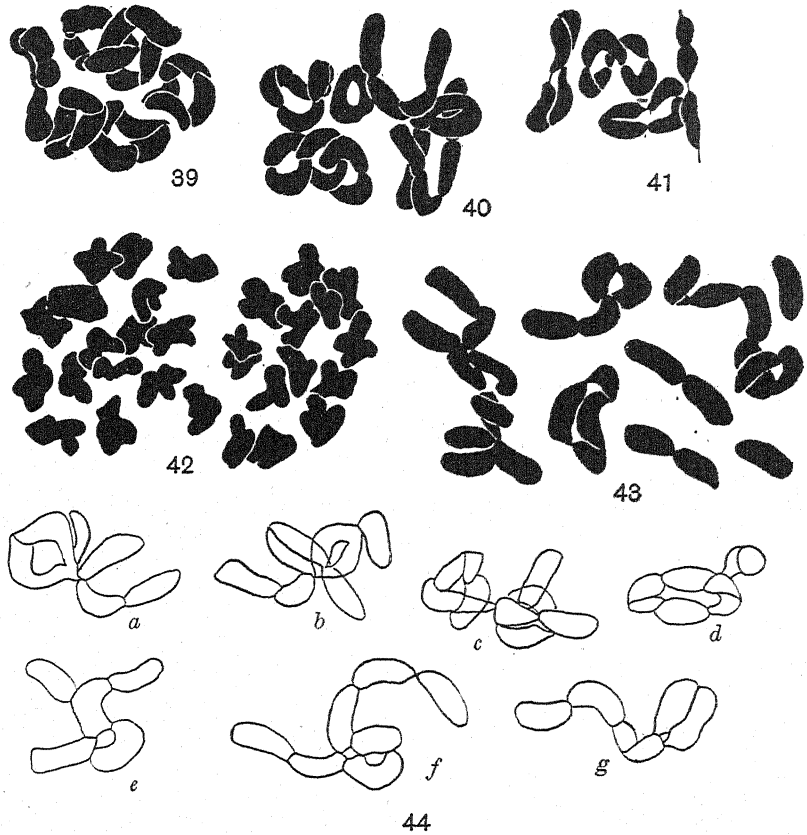
Extensive observations regarding the meiosis in different species of *Tradescantia* and especially *T. virginiana* have been recorded previously. During the present investigation the newly discovered pentaploid plant afforded a good opportunity for a comparative study of meiosis in this species with that of the tetraploid *T. virginiana* L. from which it has arisen. Earlier stages of pairing could not be studied however, due to the poor fixation obtained after following the usual cytological methods. It seems that some special procedure needs to be developed in order to obtain good fixation of earlier stages of meiosis. During diakinesis also the fairly long chromosomes forming complex multivalent associations overlap each other in such a way that it becomes difficult to find out the real connexions between the chromosomes. Cases clear enough to allow critical observations were, however, observed at places (Figs. 83, 84). It is worth recalling here the observations of Newton & Darlington (1929) in the pentaploid species *Tulipa clusiana* DC., where they remark, 'At

diakinesis the mass of chromosomes becomes too dense for useful study of the compound associations'.

According to Anderson & Sax (1936) in the diploid Tradescantias 80 % of the chiasmata are terminal, in the tetraploids all are terminal. Darlington (1929) first pointed out that the association of two or more chromosomes at one or more points forming complex configurations as generally found in the tetraploid Tradescantias was due to the terminalization of chiasmata originally formed between homologous chromosomes and terminalizing either in the same or in opposite directions. Both Darlington (1929*a*), and Koller (1932) have recorded clear cases of interstitial chiasmata in some of the tetraploid Tradescantias. Darlington (1938) has assumed that in the tetraploid Tradescantias chiasmata are formed almost near the ends of chromosomes, and by diakinesis and first metaphase they terminalize completely. During the present investigations no clear cases of interstitial chiasmata were observed either in the pentaploid or in the tetraploid forms. They were found as a rule to be terminal during diakinesis. To explain the constant appearance of terminal chiasmata in the tetraploid Tradescantias Anderson & Sax (1936) on various grounds concluded that the distal ends of the chromosomes paired early enough to insure chiasma formation there, and by the time the median regions had been paired chiasma formation was inhibited. Recently, Darlington (1939) has come to the same conclusion as Anderson & Sax, and assumes that due to time factor both chiasma formation and pairing in the tetraploid Tradescantias are mostly localized near the ends of the chromosomes. The small fragments observed associated with a dicentric chromosome and the bridge also provide indirect proof of the smallness of the inverted segment as well as the almost proximal chiasma formation in the tetraploid and pentaploid forms. It seems clear, therefore, that the terminal chiasmata observed in those complex multivalent associations in the tetraploid and pentaploid forms are primarily due to the restricted pairing of the homologous segments of chromosomes.

In the tetraploid form association of homologous chromosomes into bivalents, trivalents, quadrivalents and chains of five to eight chromosomes has been observed. The most common and predominant multivalent association was found to be rings or chains of four. Five rings of four and two bivalents have been shown in Figs. 39 and 82. Six rings of four have, however, not been observed. Formation of ring chromosomes in *Tradescantia* has been discussed previously by Darlington (1929), and Sax & Anderson (1933). In the pentaploid form, besides the univalents, bivalents, trivalents, quadrivalents and pentavalents, higher association

of chromosomes forming complex configurations has been observed frequently (Fig. 44). Due to overlapping of chromosomes and the very deceptive appearances formed thereby a statistical analysis of the frequency of occurrence of the different kinds of multivalents has not



Figs. 39–41. Plant no. 1. Fig. 39. Diakinesis; five rings of four and two bivalents. Fig. 40. Showing proximal interlocking of bivalents. Fig. 41. Showing regular disjunction and double non-disjunction on the same side of the ring of four chromosomes. Figs. 42–44. Plant no. 2. Fig. 42. First anaphase showing fifteen chromosomes at each pole. Fig. 43. Showing the association of the thirty chromosomes during diakinesis; $1_{VI}+2_{V}+1_{IV}+3_{III}+1_{II}+2_{I}$. Fig. 44 *a–g*. Complex multivalent associations involving multiple chiasmata. × 1400.

been attempted. In Fig. 43 the thirty chromosomes of the pentaploid plant in a pollen mother cell during diakinesis are drawn separately. The chromosomes are associated as one hexavalent, two pentavalents, one ring of four, three rods and one ring of bivalent and two univalents. Different types of pentavalent associations were observed, some of which

have been shown in Figs. 44 and 84. The percentage of univalents per pollen mother cell was found to be quite low. One, two, or in rare cases three, were found in a nucleus. Multivalent associations, on the other hand, were found to be more common than bivalents, and two to three pentavalent groups were commonly met with in each mother cell. In the pentaploid species *Tulipa clusiana* DC., Newton & Darlington (1929) found a much higher percentage of univalents and bivalents per pollen mother cell. This difference is probably due to the autopentaploid condition of the present pentaploid plant. It is generally accepted that pairing of homologous chromosomes cannot be complete in haploid, triploid and pentaploid organisms. The very low percentages of univalents and high percentage of pentavalents and higher associations show on the contrary that the frequency of pairing of chromosomes is quite high in the present pentaploid plant. The frequent occurrence of multivalent associations involving higher numbers of chromosomes than the chromosome number of the plant would suggest is due to the presence of reduplicated segments in non-homologous chromosomes as also shown by Catcheside (1932) in the case of haploid *Oenothera blandina.*

Proximal interlocking of bivalents has frequently been observed in the tetraploid form (Fig. 40). Sax & Anderson (1934) have found that 8·7 % of the bivalents in the twenty-eight different diploid species of *Tradescantia* show interlocking at metaphase. They are of opinion that the occurrence of interlocking bivalents in these diploids is probably correlated with segmental interchange between non-homologous chromosomes. According to Catcheside (1932) the high percentage of interlocking found in many species of *Oenothera* was due to the entangling of the pairing threads of zygotene.

(ii) *Non-disjunction*

In spite of the high percentage of multivalent formations, the disjunctions of chromosomes during first anaphase is fairly regular in the tetraploid plant. In the triploid and in the pentaploid plants the percentage of non-disjunction was found to be very high (Table 6). The actual percentage of non-disjunction is little higher than the figures presented in Table 6 because the present data were obtained from dividing nuclei only. Besides the general type of non-disjunction leading to the formation of pollen grains with different chromosome numbers another type of non-disjunction was observed. In this latter type instead of alternate chromosomes in the ring of four passing to opposite poles two adjacent chromosomes in the ring of four move to the

same pole as shown in Fig. 41. Thus pollen grains with the same chromosome number but different genetic constitutions are formed. If nucleolar chromosomes are involved in such a type of irregular disjunction, the origin of pollen grains with different combinations of sat.-chromosomes and nucleoli than normally expected may easily be foreseen. Non-disjunction of homologous chromosomes has a marked effect on the fertility of the pollen grains in the case of the triploid plant, but in tetraploid and pentaploid forms the correlation is very low. This is shown in Table 6. An explanation regarding the relative survival chances of pollen grains with different chromosome numbers has been presented earlier in this paper.

Table 6

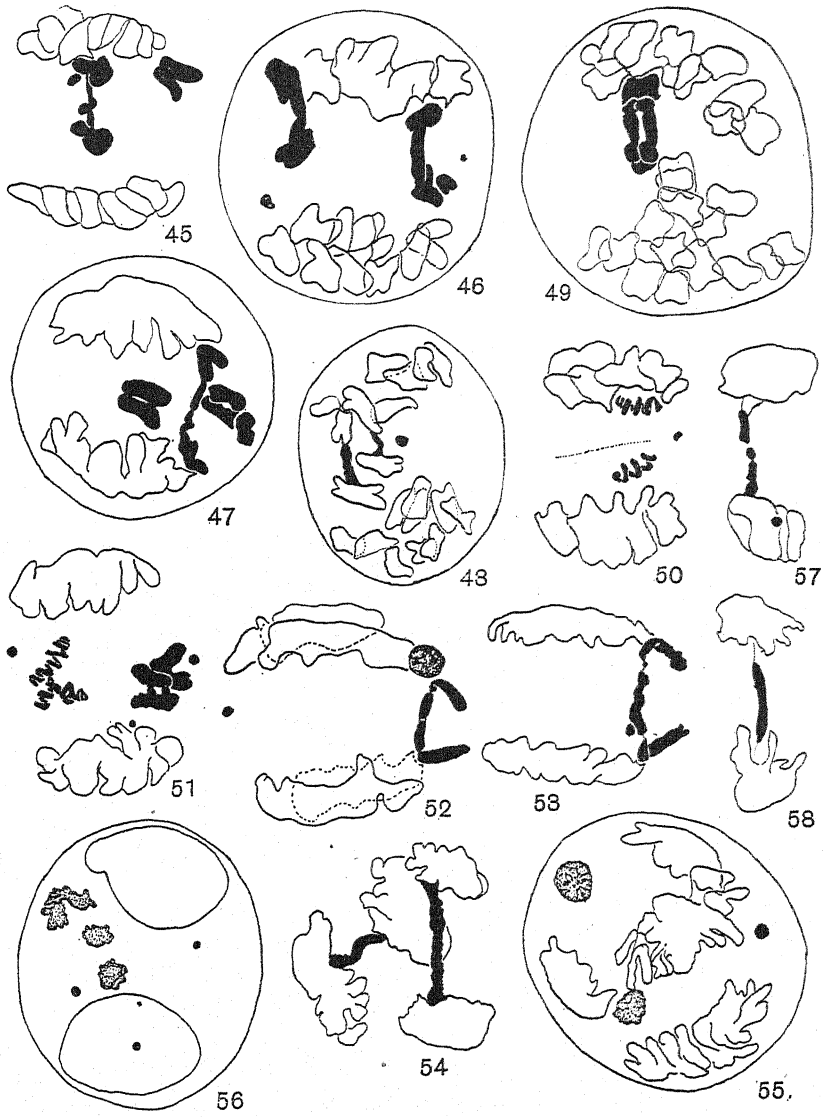
	Triploid *T. brevicaulis*	Tetraploid Plant no. 1	Pentaploid Plant no. 2
Percentage of non-disjunction	70	30	65
Percentage of abortive pollen grains	55	15	20
Percentage of bridge formation:			
1st division	—	12	11
2nd division	—	None	15
Percentage of lagging chromosomes, with or without fragments	—	5	28
Percentage of extruded spherical bodies in healthy pollen grains	24	6	20

(iii) *Lagging chromosomes and the origin of the extra nuclear bodies*

Lagging bivalents were frequently observed during first anaphase. They have been found more frequently in the pentaploid than in the tetraploid plant (Figs. 45, 47, 51) (Table 6). Lagging univalents were, however, very rarely observed in the pentaploid plant. This corroborates the previous observation that the number of univalents per pollen mother cell is very low in this plant. According to King (1933), the triploid *Tradescantia* spp. he examined showed a large and variable amount of lagging chromosomes during first anaphase and telophase. The lagging chromosomes often failed to reach the pole and formed micronuclei (Figs. 52, 55, 56). According to Sax (1937) the lagging chromosomes at meiosis in the triploid *T. bracteata* include univalents which usually divide equationally, and chromosomes which are dicentric or are retarded by the chromatid bridge. During the present investigation on the tetraploid and pentaploid plants, it was found that these lagging bivalents generally fail to disjoin, and even if they disjoin the disjunction takes place so late that they seldom reach the poles or get included in the nucleus. During second division these bivalents undergo a certain amount of telophasic metamorphosis and form micronuclei. Still later they appear

as densely stained spherical bodies in the tetrads or the pollen grains. These bodies are Feulgen-positive (Figs. 52, 55, 56, 80, 89). According to Darlington (1938), these spherical extra-nuclear bodies in tetraploid Tradescantias are formed entirely by the lagging chromosomes. In the tetraploid and the pentaploid plants, mostly one, rarely two or three such bodies were observed in the pollen grains. In the triploid, however, more than one such body in a pollen grain was frequently observed. A percentage correlation between the lagging chromosomes and these spherical bodies in the tetraploid and pentaploid plants has been presented in Table 6. In the triploid species 30 % of the normal pollen grains showed the presence of these bodies. The actual percentage must be much higher, as degenerated and deformed pollen grains with such bodies are not included in the calculation. Although pollen mother cells were not examined in this triploid plant, it can be assumed that these bodies are only formed from lagging bivalents or univalents. The variation in size of these bodies in the pollen grains indicates whether they are formed from a bivalent or a univalent lagging chromosome; this variation in size is well marked. It is worth recalling here the observations of King (1933), who found that out of one hundred pollen mother cells showing late anaphase fifty-nine showed one to three lagging chromosomes. Myers & Hill (1941) also found in the case of some grasses that the micronuclei are formed entirely from lagging chromosomes. They have explained further that, assuming two lagging bivalents per pollen mother cell during first anaphase, only two pollen grains in a tetrad may show the presence of one such body. It follows, therefore, that the percentage of pollen grains with these bodies will be always less than the percentage of pollen mother cells showing lagging chromosomes. According to King (1933) pollen sterility in the triploid *Tradescantia* spp. studied was primarily due to chromosome deficiency resulting from lagging at meiosis. Sax (1937) also holds the view that the loss of chromosomes by lagging at meiosis accounts for some of the pollen sterility in *Tradescantia* spp. From Table 6, as well as from the above account, it will be noted, however, that presence of these bodies in the pollen grains does not indicate non-viability of the pollen grains. Healthy and actively dividing pollen grains containing such bodies were most frequently met with in the triploid, tetraploid and the pentaploid plants (Fig. 80). The correlation between non-disjunction and pollen sterility has already been discussed earlier in this paper.

The lagging bivalents although generally failing to reach the pole were sometimes seen disjoining. Due to the delay, however, the univalents



Figs. 45–58. Showing inversion bridges, fragments, lagging chromosomes and micronuclei formation in the pollen mother cells of plant no. 1 and plant no. 2. Figs. 45, 46, 48 and 56 are from plant no. 1, the rest are from plant no. 2. Figs. 45, 46, and 48. Inversion bridges and fragments during first division. Fig. 47. Lagging bivalents and a univalent. Fig. 49. Bivalent bridge during first anaphase. Figs. 50, 51. Despiralization of lagging bivalents. Figs. 52, 53, and 57, 58. Inversion bridges and fragments. Note that the points of break of the bridge do not necessarily indicate points of maximum stretching or torsion. Fig. 54. Second division bridge. Figs. 55, 56. Formation of micronuclei. × 1400.

are not included in the nuclei and form the typical micronuclei and the spherical extrusion body seen later on. Despiralization of the univalents in a lagging bivalent as observed in few cases (Figs. 50, 51, 88) indicates clearly that this process is the result of the reaction between the chromosomes and the altered physical properties of the cytoplasm and can take place irrespective of the polar separation or formation of a nuclear membrane. Micronuclei with nucleoli were seldom found, which shows that lagging bivalents seldom involve nucleolar chromosomes.

(iv) *Inversion bridges*

Quite a high percentage of first division bridges has been observed in both the tetraploid and the pentaploid plants. It was found also that while second division bridges were absent in the tetraploid, in the pentaploid plant their percentage was higher even than the first division bridge (Table 6). Upcott (1937) has classified different species of *Tulipa* into three sections according to whether the percentage of first division bridges in a species is more than 10 % of the cells; less than 10 % of the cells or bridge formation is altogether absent in the species. According to the above classification, both the tetraploid and the pentaploid forms come under the first group showing a significant percentage of bridge formation. It cannot be said at present whether bridge formation in other varieties of *Tradescantia virginiana* is equally frequent. It is interesting to compare the observations of Darlington on this point. He states: 'The inversion crossing over is most frequent in the diploid species of *Tradescantia*, occurring in over 1 % of cells. It is least frequent in the tetraploids and in *Rhoeo*'. Sax (1937) found that bridge formation in the triploid species of *Tradescantia* was higher than in the diploid. In the diploid species it varied from less than 1 % to nearly 5 %, whereas in the triploid (*T. bracteata*) it was 16·3 %. He maintains, however, that the great increase in dicentric chromatids in the triploid is not caused by a greater number of inversions per chromosome than are present in the closely related diploid species but is related to the type of chromosome association at meiosis.

The bridges at both the first and second anaphases consist mostly of a dicentric chromatid and an acentric fragment (Figs. 45, 46, 48, 52, 53, 85, 86). Due to the second division split already present at the first anaphase, it appears sometimes as if the other chromatids of the bivalent are also involved in the bridge on each side (Fig. 47). The small size of the acentric fragment at once indicates that not only is the inverted segment very small, but also the cross-over in the inversion region is

near the proximal end of the chromosome. Generally one bridge was observed during both first and second anaphases, but two bridges have also been observed (Figs. 46, 48). Formation of a bivalent bridge has been shown in Fig. 49. It was found that while in the tetraploid plant there was a close percentage correlation between the bridges and the acentric fragments, in the pentaploid plant, on the other hand, the percentage of fragments was much higher than the bridges. Fragments were only observed during first anaphase onwards and could not be traced during first metaphase or diakinesis. In the pentaploid plant nearly 50 % of the pollen mother cells showing first anaphase or telophase showed the presence of a fragment, but during the second division and the tetrad stages more than 90 % of the cells were found to have at least one fragment per cell. Generally there is only one fragment per pollen mother cell. Two fragments per pollen mother cell are not infrequent, and occasionally three fragments were observed (Figs. 51, 56). These fragments generally lie in the cytoplasm and are variable in size. The fragments at the first division generally appear as slightly bigger than those observed during the second divisions. They are very seldom traceable in the pollen grains. Sax (1937) found that half of the dicentric chromatids in the *Tradescantia* spp. are not accompanied by acentric fragments. He believes that the heterozygous inversions are very small and near or at the ends of the chromosome arms, and hence the acentric fragments produced from such inversion cross-overs are too small to be seen. According to Darlington (1938) in the tetraploid Tradescantias and in *Rhoeo* the fragment is often invisible. This he has explained as due to either the fragments being lost in the general body of chromosomes or they are simply too small to be seen owing to the inversions lying very close to the ends of chromosomes. Emsweller & Jones (1937) have also found a much higher number of fragments in the pollen mother cells than the number of bridges would suggest. They concluded, therefore, that fragments may be formed without involving bridge formation. Emsweller & Jones found that the frequency of pollen mother cells with fragments remains practically constant during first and second divisions. During the present observation on the pentaploid plant it was found, however, that not only does the frequency of pollen mother cells with fragments double during the second division, but more than 90 % of the pollen mother cells show the presence of fragments. As these fragments were not observed before first anaphase, it appears that they are detached from the body of the chromosomes by the movements leading to the separation of the chromosomes. Doubling of the number of fragments

during second division suggests at once that these fragments also undergo equational division. Though actual divisions of the fragments were not observed the difference between the sizes of the fragments observed during first and second divisions respectively strongly suggests the probability of equational division of the fragments. It may be suggested here that those fragments which cannot be correlated with the bridge formation represent inverted segments in the chromosomes which fail to pair due to mechanical interference and are eliminated during divisions of chromosomes.

(v) *Spontaneous breakages of chromosomes*

Due to the close resemblances between the structural changes of chromosomes produced by X-rays and those found to occur spontaneously in nature, the subject has received great attention in recent years. Giles (1940) has described the various kinds of such changes occurring spontaneously in different species and hybrids of *Tradescantia*. Darlington & Upcott (1941) recently published a comprehensive account on the subject and have been able to classify the various kinds of structural alterations of chromosomes in different species and hybrids of *Tulipa*, *Tradescantia* and *Fritillaria*. During the present observations on *Tradescantia* spp. some spontaneous chromosome aberrations were also observed. Contrary to the observations of Giles (1940), instead of chromatid breaks chromosome breaks were mostly observed (Figs. 62–64, 89). The formation of dicentric chromosomes and acentric fragments during first and second divisions in the pollen mother cells of the tetraploid and pentaploid plants of *T. virginiana* have already been described. Sax (1940) has shown that a dicentric bridge with acentric fragment may arise during the pollen-grain division without involving heterozygous inversion—cross-over. Though Giles (1940) has observed such structural changes of chromosomes during the first microspore division in different species of *Tradescantia*, during the present investigation bridge formation in the microspores was not observed.

It is generally accepted that gametes with the structurally altered chromosomes produced from such dicentric chromosomes seldom survive, and therefore pollen grains showing the presence of such altered chromosomes are rarely met with. For this reason also we find generally a correlation between the frequencies of bridge formation and sterile pollen grains in a species. Clear cases were observed, however, showing the persistence of such altered chromosomes in the pollen grains. In Figs. 63 and 90, for instance, the chromosome with subterminal constriction

represents most likely one of the broken halves of a dicentric chromosome. If a dicentric chromosome is included in the pollen-grain nucleus

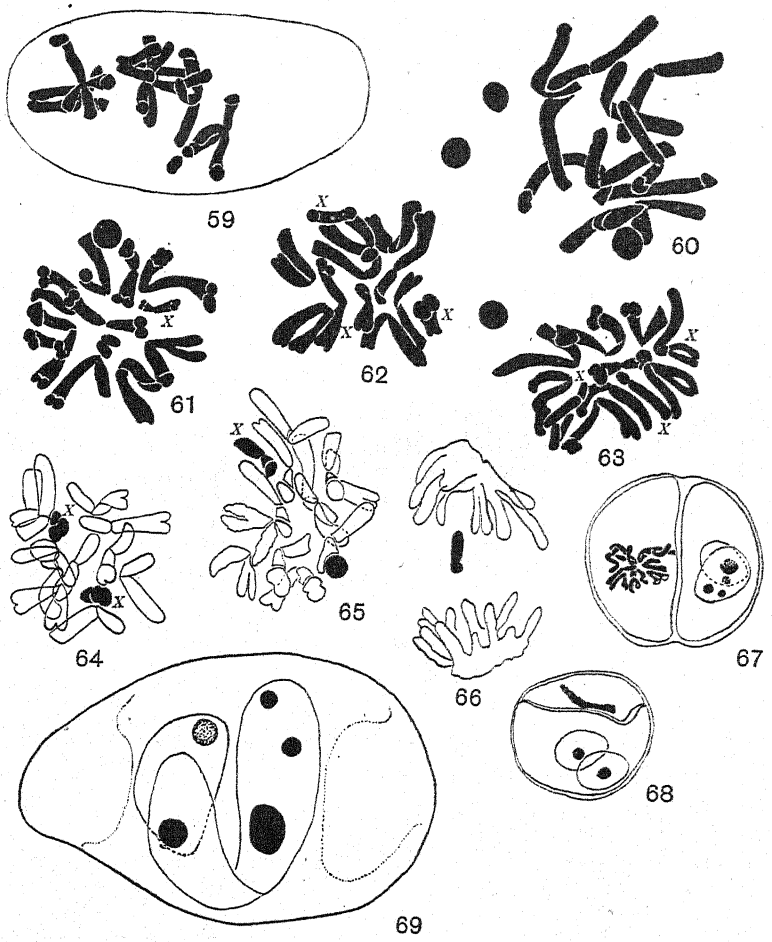


Fig. 59. Abnormal pollen grain in *T. brevicaulis* with six chromosomes dividing precociously. Fig. 60. Abnormal growth of a deficient pollen grain ($n=10$) of *reflexa*; note the extra long size of the chromosomes and three spherical chromatic bodies. Figs. 61–65. Spontaneous chromosome aberrations in pollen grains. The chromosomes showing structural changes are marked *X*. Fig. 61. *montana*. Fig. 62. *T. brevicaulis*. Fig. 63. *reflexa*. Figs. 64, 65. Plant no. 1. Fig. 66. Elimination of a big fragment of chromosome during second division in a pollen mother cell of plant no. 2. Figs. 67, 68. *reflexa*; showing abnormal cytokinesis. Fig. 69. *montana*; showing abnormal growth of the vegetative nucleus. × 1400.

instead of forming a bridge and subsequently breaks inside the nucleus, two short chromosomes with subterminal constrictions may be formed. These chromosomes will naturally fail to grow normally and will appear

as deformed chromosomes. Similar instances have been observed in the pollen grains of the triploid *T. brevicaulis*, the tetraploid *T. virginiana* U.S.A., and in the pentasomic form *montana* (Figs. 61–65). Chromosome break in one arm of a V-shaped chromosome, liberating a big acentric fragment, has been observed in the pentaploid plant during the second meiotic anaphase (Fig. 66). Such breaks may also transform a V-shaped chromosome into a shorter chromosome with submedian or subterminal constriction. Breaks or actual fractures in the arms of a metaphase chromosome in one or more points have been frequently observed in the pollen grains of *T. brevicaulis*, *reflexa*, *caerulea* and especially in the pentasomic form *montana*. These chromosome breaks are quite distinct from the others already described. The broken fragments generally lie very close to the broken arms of the parent chromosomes, and sometimes they were found still attached to the parent metaphase chromosome (Figs. 63, 89). It shows that although the real chemical disunity may take place earlier in the nucleus, actual separation revealing such a break can only be recognized during separation of the chromosomes. Darlington & Upcott (1941) found different kinds of chromosome alterations in a triploid species of *T. virginiana* from the Lyons Botanical Garden. They did not find, however, such structural changes in the pollen grains of the triploid form *T. brevicaulis*.

Although pollen grains with such structurally altered chromosomes have been found in the divisional stages, their subsequent development, viability and practical genetic value is a doubtful matter. It is worth pointing out here also, that these markedly altered conditions of the chromosomes were mostly met with only in those pollen grains showing an unbalanced chromosome complement and most likely abnormal metabolic conditions.

(vi) *Other irregularities in microsporogenesis*

Two fully formed pollen grains locked within a mother cell in various stages of division have been observed frequently in forms like *reflexa*, *caerulea*, *montana*, as well as in the tetraploid plant no. 1 (Fig. 67). It is difficult to say whether these represent dyads which later develop into two pollen grains or are simply formed as a result of incomplete separation of the tetrads. In the latter case each cell represents a half-pollen mother cell. The size of these cells containing two pollen grains suggests, however, that each represents one full pollen mother cell. These two pollen grains are generally liberated later from the mother cell and become indistinguishable from the rest. Sometimes four microspores were seen locked

in a mother cell, two or three of which show degeneration (Fig. 68). It is well known that partial or complete failure of cytokinesis may lead to the formation of polyploid pollen grains. The giant pollen grains observed in plant no. 1 and in the form *reflexa* (Fig. 91) are most likely developed from the fusion of two adjacent nuclei locked in the pollen mother cell. These giant pollen grains were also found in divisional stages. Definite information regarding their viability cannot be given at present. Different kinds of irregularities in the growth and differentiation of the pollen grains have also been observed. Non-differentiation of the generative and vegetative nuclei was commonly met with. Sax (1937) has also found this type of non-differentiation in the triploid *T. bracteata*. Abnormal growth of the vegetative nucleus (Fig. 69), amitotic-like divisions of both the vegetative and generative nuclei and various shapes of vegetative and generative nuclei were frequently observed.

Asymmetrical orientation of the spindle and flattening of that pole in contact with the pollen wall was seen during first microspore division in all the species of *Tradescantia*. This appearance is produced as a result of stretching of the spindle against the mechanical resistance of the wall of the pollen grain (Darlington, 1938). The orientation of the spindle during first division of the pollen grains shows a constant and distinct polarity; as a result of this the generative nucleus is always so placed as to be restricted to the narrowest part of the pollen grain and against the wall or simply adjacent to the wall according to the shape of the grain. A marked polarity in the orientation of spindles in the female gametophytes of higher plants is a well-known character (Bhaduri, 1935). Due primarily to the combined effects of the mechanical interference of the pollen wall and the extension of the spindle, the generative nucleus not only becomes morphologically differentiated from the vegetative nucleus but also fails to assume the dispersed condition seen in the resting stage of the vegetative nucleus.

4. DISCUSSION

The present observations have clearly indicated the close correspondence between the number of sat.-chromosomes on the one hand and the maximum number of nucleoli on the other in both root-tip cells and microspores of different species of *Tradescantia*. The genus *Tradescantia* provides also a good example of how polyploidy may bring about a corresponding increase in number of the nucleoli and sat.-chromosomes. De Mol (1928) first suggested that the number of nucleoli generally represents the number of genoms present in a species. In *Hyacinthus* spp.

he found that haploid cells have one, diploids two and triploids three nucleoli. Although this generalization has been found to hold good in the majority of cases, quite a number of observations have been recorded from time to time invalidating the theory. For instance, a number of well-defined diploid plants have been found to possess, instead of two, four or more nucleoli in their somatic cells (Bhaduri, 1940; Sikka, 1940; Iyenger, 1939; Sinotô, 1938; Sato, 1939; Pathak, 1940*a*). In a few of such anomalous cases, it has been interpreted on various grounds that these species, generally known as diploids, are really allotetraploid species (Nandi, 1936; Gates, 1939; Iyenger, 1939; Sikka, 1940; Pathak, 1940*a*). There are, however, still other cases where the presence of more than two nucleoli in a diploid species cannot be interpreted on the basis of secondary polyploidy.

Another kind of evidence which disproves the rule of correspondence between the number of nucleoli and the number of genoms present in a species has been put forward by McClintock (1934) in the case of *Zea mays*. She observed that a segmental interchange at the region of the nucleolus organizer of the sat.-chromosome (chromosome 6) with another non-nucleolar chromosome (chromosome 9), produces two morphologically different nucleolar chromosomes, one with a satellite and the other with a portion of the nucleolus organizer and a secondary constriction at the point of interchange. She has been able to show that homozygous recombinations of gametes which had such interchanged chromosomes produced those plants in the culture showing four nucleoli, instead of two, in their somatic nuclei. This is, at present, the only experimental evidence indicating how increase of the nucleolar number in a species may take place by structural changes of chromosomes.

Turning to the problem in the case of *Tradescantia* spp., we are confronted with the same difficulty, namely, how to interpret the presence of two nucleoli and two sat.-chromosomes in the haploid complement. It is necessary to decide whether this condition is an expression of secondary polyploidy or has arisen from segmental interchanges of chromosomes similar to those described by McClintock (1934). Cytological evidence in general does not support the view of considering a diploid species of *Tradescantia* such as *T. crassifolia* as a secondary polyploid species. A number of the diploid species of *Tradescantia* have triploid and tetraploid forms growing in nature. Cytological evidence shows that interchange of segments between non-homologous chromosomes must have taken place in the nuclear evolution of the diploid species of *Tradescantia* (Anderson & Sax, 1936). It is still very difficult to interpret, for instance, on the

general principles of segmental interchange of chromosomes the evolution of the two types of sat.-chromosomes in *T. crassifolia*. Slight modification of the generally accepted principles of segmental interchange seems, however, to provide a probable explanation of the nature of those structural changes of chromosomes which may have produced the two sat.-chromosomes in the gametes of *T. crassifolia*. The theory put forward below also explains the origin of the so-called 'fragments' in *Tradescantia* spp.

It is generally assumed that during an interchange between two chromosomes, each chromosome receives a segment from the other. It is also assumed that broken ends of chromosomes always tend to unite freely one with another. Union of a segment or interchange at the free end of an unbroken chromosome is not only rare but also doubtful. It is quite obvious that the possibility for chemical union between two different chromosomes at two different points depends on the chemical affinity between two such points. There is every reason to assume that the region responsible for the organization of the nucleolus in a sat.-chromosome has slightly different chemical properties from the rest of the body of the chromosome. It is easy to conceive, therefore, that following a break at this particular region the freedom for chemical union at such a point would be more restricted than at any other point in the body of the chromosome. In other words, whereas a broken chromosome segment may unite freely with any other broken ends, if there is a break at the locus of nucleolus organization of a sat.-chromosome, the broken end of the sat.-chromosome would not freely unite with just any portion of a non-satellited chromosome. *T. crassifolia*, as already described, has two sat.-chromosomes, one with a big head and conspicuous filament, and the other with no head but a minute thread only. The probable origin of these two chromosomes by a process of translocation and loss of a segment between a sat.-chromosome and a non-sat.-chromosome is shown diagrammatically in Fig. 70.

Before describing the result of such an interchange of segments, let us first assume a break at the point X in the region of nucleolus organization of the sat.-chromosome AS and a corresponding break at the point Y of a non-nucleolar chromosome OP. Both the chromosomes have median primary constrictions. The broken end of the segment OY being more free to unite links up with the broken segment S, whereas the segment AX having a restricted freedom for chemical union fails to unite with the segment P. Thus we have produced a free fragment P, the chromosome OYS with a satellite and chromosome AX with a remnant

of the satellite filament. The new chromosome *OYS* having a proper satellite and the chromosome *AX* still retaining a portion of the satellite thread, each in turn continue to organize a nucleolus. As is always the case, such changes in the structure of the chromosomes must be assumed to have taken place during premeiotic divisions or in the spore mother cells. Homozygous recombination of gametes with such structurally altered chromosomes may produce plants with four sat.-chromosomes and four fragments in their somatic cells or two sat.-chromosomes in the gametes, one with a conspicuous satellite and sub-median primary constriction and another with a minute filament and median primary constriction. The condition of the sat.-chromosomes and nucleoli described for *T. crassifolia* tallies almost with the description given above. The regular and mostly normal separation of these fragments

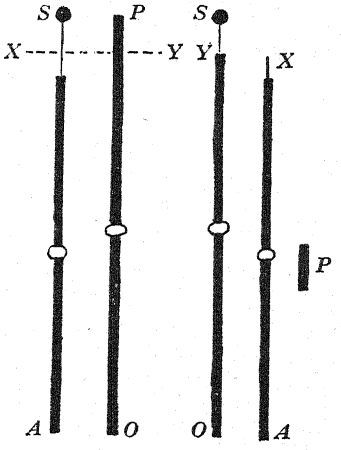


Fig. 70.

during mitosis shows that they behave as independent chromosomes. Subsequent differentiation of an almost terminal primary constriction in each such fragment may be reasonably assumed (cf. Koller, 1932). If the present hypothesis be correct, it follows that the filament of the satellite really represents the chemically differentiated locus for the organization of a nucleolus, and not the base of the satellite as McClintock (1934) has suggested. It may be pointed out here, however, that the process of organization of a nucleolus may commence from the base of the satellite as suggested by Gates & Pathak (1938).

The common spiderwort, *T. virginiana* L., has been grown in Europe for more than three hundred years (Anderson & Sax, 1936). A number of *Tradescantia* spp. having both diploid and tetraploid forms are known

to occur in nature. The occurrence of diploid species of *Tradescantia*, however, has not been reported from Europe. Though a diploid form of *T. virginiana* has not yet been discovered it is generally accepted that this species is autotetraploid. The results of the analysis of sat.-chromosomes during the present investigation made this assumption no longer tenable. The presence of three types of sat.-chromosomes at once brings doubt as to its autotetraploid nature even if we assume segmental interchanges of chromosomes after duplication. If we assume that the diploid parent had either a pair of sat.-chromosomes similar to that found in *T. crassifolia* or one sat.-chromosome and another chromosome with a secondary constriction, simple segmental interchange is not sufficient to explain the transformation of four such chromosomes into the three sat.-chromosomes and the chromosome with secondary constriction as present in *T. virginiana*. The only adequate method of interpretation is to assume

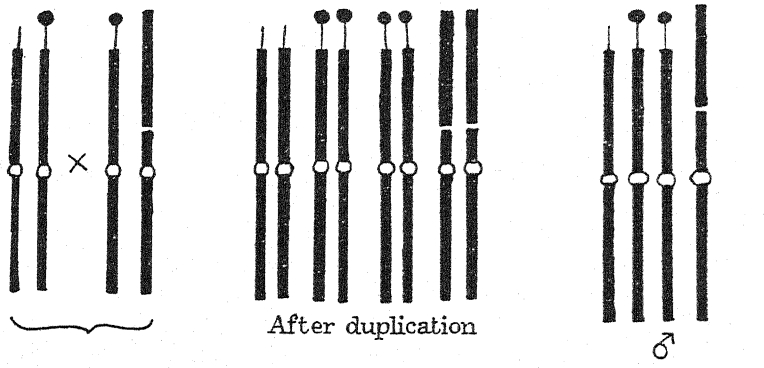


Fig. 71.

a cross between two different gametes and the subsequent doubling as shown diagrammatically in Fig. 71. As analysis of sat.-chromosomes has not been made in any of the related diploid species except the quite different species *T. crassifolia*, the presence of the other type of sat.-chromosome has to be assumed.

The nature of the association of chromosomes during diakinesis in the pollen mother cells of the tetraploid plants contradicts, however, the amphidiploid or allotetraploid derivation, because the chromosomes instead of forming bivalents generally form rings or chains of four. It must be pointed out here, however, that the related diploid species also show a ring of four chromosomes during diakinesis.

The constancy of nucleolar sizes in different species has been reported frequently. From recent observations (Bhaduri, 1939, 1940, 1941)

it can be taken in general that like the constancy of the size and shape of chromosomes in a complement, the *size relation of nucleoli* in a species is a constant or specific character. The present observations in *Tradescantia* not only corroborate the above generalization but shows also that structural changes of chromosomes may bring about changes of the size relation of nucleoli in a species. As a workable hypothesis it may be assumed now that a true diploid species has a pair of homologous sat.-chromosomes and correspondingly a pair of homologous and identical nucleoli in the somatic nuclei. A change in the number of nucleoli in a species can be brought about by polyploidy as well as by segmental interchanges between sat.-chromosomes and non-nucleolar chromosomes. Increase in the nucleolar number by such structural changes of chromosomes not only alters the morphology of the affected chromosomes but may also alter the relative size of the nucleoli. According to the above hypothesis, therefore, presence of two different pairs or four different nucleoli in the somatic nuclei of diploid species, without showing evidence of secondary polyploidy, has to be interpreted as due to structural changes of chromosomes, brought about by segmental interchanges between satellited and non-satellited chromosomes in the course of the nuclear evolution of the species in question. Corroboration of such a previous interchange may be obtained from the nature of association of chromosomes during diakinesis. Exceptional conditions have already been found in *Oenothera*. The homozygous species like *O. Hookerii*, which according to several authors form seven free bivalents during diakinesis, have two dissimilar pairs of nucleoli in the somatic nuclei (Bhaduri, 1940). There is at present no definite evidence of the species being a secondary polyploid. Further, cytological evidence does not indicate any previous segmental interchanges between non-homologous chromosomes. Before coming to any definite conclusion on this case, it is necessary in the light of the present observations to make further cytological examinations of different homozygous species of *Oenothera* and other closely related genera. Further, the generally accepted assumption that non-homologous interchanges always lead to ring formation during diakinesis needs to be reconsidered.

According to Sax (1937), the origin of the triploid species *Tradescantia bracteata* has been interpreted as due to crossing of a diploid gamete with a haploid one. Cytological analysis of the triploid species *T. brevicaulis*, on the other hand, does not show autotriploid condition. The presence of three types of nucleolar chromosomes and nucleoli as observed in the tetraploid species *T. virginiana* clearly indicates an allo-

triploid condition. It has been mentioned before that material for observation was obtained from a solitary plant growing at Kew Gardens for many years. The exact history of this plant is not known. It has also been pointed out before that the occurrence of diploid species related to *T. virginiana* has not been reported from Europe. It seems highly probable, therefore, that the triploid species *T. brevicaulis* arose as a natural cross between two different diploid and tetraploid species. The plant at Kew has most probably been raised from seeds originally imported from America.

The origin of the pentaploid species (plant no. 2) is also very difficult to explain. The exact history of the introduction of this plant to the Botanical Garden of Bristol University is not available. Cytological observations indicate clearly that it is an autopentaploid species. The possible origin from a cross between $3n$ and $2n$ gametes is highly improbable because hexaploid species are not yet known. Further, development of $3n$ gametes in the tetraploid species has not been observed, though actively dividing giant pollen grains, which are tetraploid as a rule, were found in the anthers of the tetraploid species. The probability of a union between such a tetraploid giant pollen grain and a haploid egg of some related diploid species is remote. Navashin, M. (1925) has however suggested that the pentaploid mutation which he found in a culture of 150 seedlings of *Crepis capillaris* L. arose as the result of a successful cross between a tetraploid and a haploid gamete. The probable origin of this autopentaploid plant as a somatic bud mutation from the tetraploid *Tradescantia virginiana* during the process of vegetative propagation commonly practised in horticulture must not be overlooked.

5. SUMMARY

Cytogenetical reinvestigations have been made of the following species, subspecies and varieties of *Tradescantia* with the aid of improved technique: *T. crassifolia*; *T. virginiana* L., U.S.A.; *T. brevicaulis*; *T. virginiana* L. (pentaploid); *T. virginiana* L., *montana*, *caerulea*, *lilacina*, *reflexa* and *alba*; and *T. blossfeldiana*.

The chromosomes in each of the species and varieties studied have been classified according to their sizes and the relative positions of the primary and secondary constrictions. The satellites in *Tradescantia* spp. are found to be extremely minute and dimorphic. The first type consists of a prominent head and a conspicuous filament whilst the second type is represented by a minute filament only. Satellites in such difficult material as the *Tradescantia* spp. should be searched for during prophase instead

of metaphase. Following the fuchsin-light-green technique the satellites appear as magenta-coloured bodies sharply defined against the green background of the nucleolus and thus much more easily picked out. A chromosome with a secondary constriction very near the primary one is present in the haploid complement of the triploid, tetraploid and the pentaploid forms.

A close correspondence between the maximum number of nucleoli on the one hand and the number of satellites and secondary constrictions on the other has been observed in each of the species and varieties of *Tradescantia* studied. The number of chromosomes attached to nucleoli during prophase will as a rule indicate the maximum number of nucleoli normally present in that nucleus. Evidence has been put forward that the maximum number and the relative size differences of nucleoli are specific characters and may be altered through polyploidy, hybridization and structural changes of chromosomes. In the light of the present observations the presence of four nucleoli instead of two in the diploid, eight instead of four in the tetraploid, and ten instead of five in the pentaploid species of *Tradescantia* has been interpreted as due to segmental interchanges between nucleolar and non-nucleolar chromosomes during the evolutionary history of the species and not as due to secondary polyploidy. The nature of this segmental interchange between nucleolar and non-nucleolar chromosomes altering the karyotypes of the species has been presented schematically. The origin of the polyploid forms of *Tradescantia* spp. has been discussed in general.

A study of the correlation between chromosome number, pollen-grain size, cell and nuclear volumes in the polyploid forms of *T. virginiana* showed that pollen grains with one or more chromosomes less than the normal complement induce abnormal metabolic conditions and tend to produce abortive grains depending in quantity on the nature and degree of the chromosome deficiency. On the other hand, increase in the number of chromosomes above the normal complement, within certain limits, does not affect the healthy growth of the grains. There is a direct correlation between chromosome number and cell size within closely related species.

A comparative account of meiosis of the tetraploid and the pentaploid forms of *T. virginiana* has been presented. Chiasma formation is restricted to the ends of the chromosome as may be also deduced by the smallness of the fragments observed in relation to first and second division inversion bridges. The low percentage of univalents and the high percentage of complex multivalent formations involving multiple chiasmata per pollen mother cell of the pentaploid form indicate a high degree

of pairing between duplicated homologous segments. Percentage counts of inversion bridges and fragments in the pollen mother cells of the pentaploid form indicate that the acentric fragments divide equationally during second division. Correlation between lagging chromosomes, inversion bridges, acentric fragments, micronuclei and abortive pollen grains is discussed and the data presented in a tabulated form.

Different types of spontaneous chromosome aberrations, chiefly of the nature of chromosome breaks, have been described. Irregularities in tetrad formation and divisions of the microspores are recorded.

In conclusion, I wish to express my thanks to Mr C. S. Semmens of the Botany Department for kindly taking the photomicrographs and for helpful discussions. My thanks are also due to the authorities of the Kew Gardens for granting me facilities for the collection and preparation of materials.

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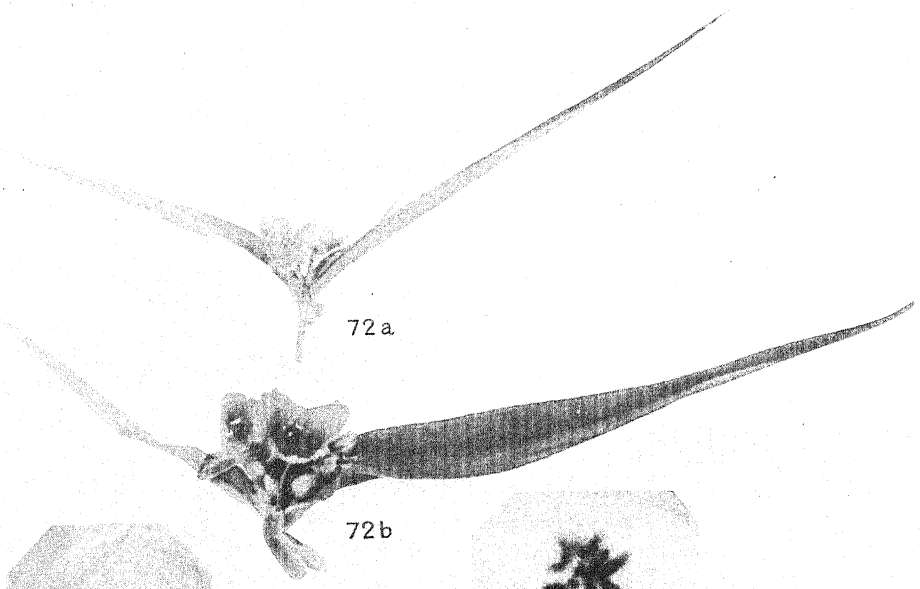

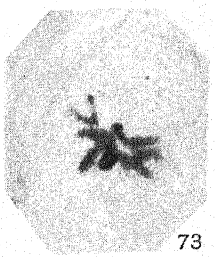
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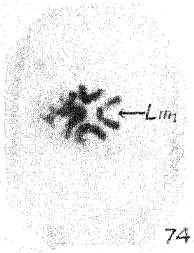

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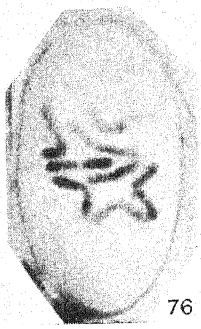
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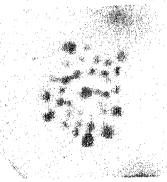
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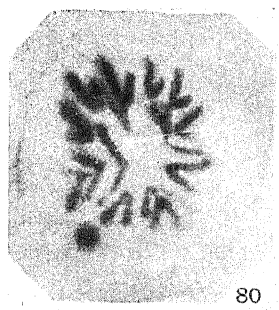
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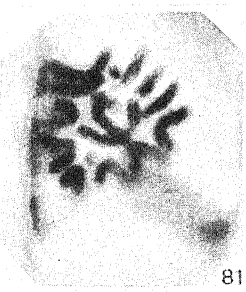
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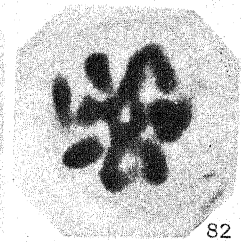
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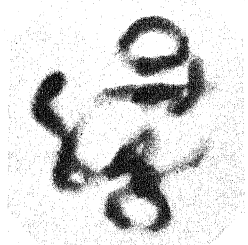
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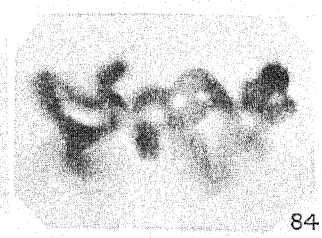
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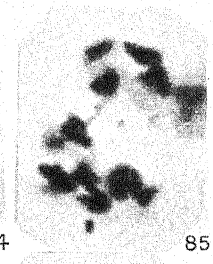
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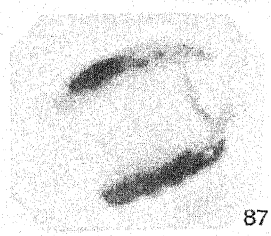
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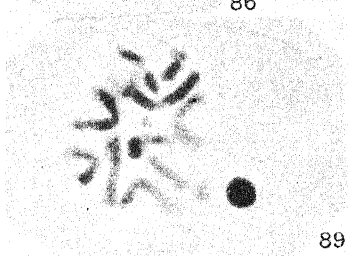
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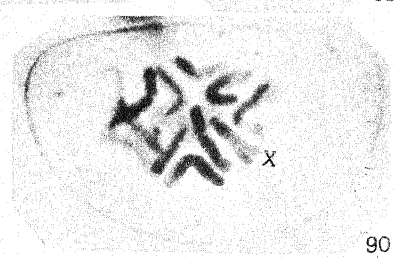

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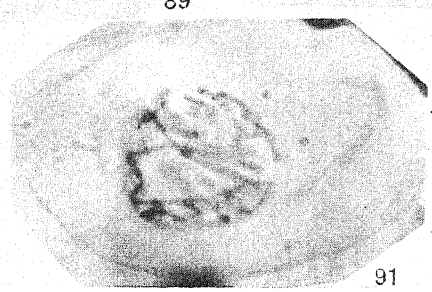
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EXPLANATION OF PLATES 4 AND 5

Excepting Fig. 72 all the figures are microphotographs taken with the aid of a Zeiss's 'Phoku' apparatus. Initial magnification ×1000.

Fig. 72*a*. Plant no. 1.

Fig. 72*b*. Plant no. 2.

Figs. 73–75. *T. crassifolia*; pollen-grain division showing the sat.-chromosome (St_1) and the size difference of the chromosomes.

Figs. 76, 77. *T. brevicaulis*; pollen grain division with eight and nine chromosomes respectively.

Fig. 78. Plant no. 1. Pollen grain with twelve chromosomes.

Fig. 79. First metaphase in the pollen mother cells of *T. blossfeldiana* with thirty-five bivalents. Note the size difference of the chromosomes.

Fig. 80. Plant no. 2. Pollen grain with sixteen chromosomes and the extra nuclear body.

Fig. 81. *Caerulea*; pollen grain with thirteen chromosomes and three fragments.

Fig. 82. Plant no. 1. Diakinesis showing five rings of four and two ring bivalents.

Fig. 83. Plant no. 2. Complex multivalent association of chromosomes, one ring of four quite clear.

Fig. 84. Plant no. 2. Two pentavalent associations. Compare fig. 44*d*.

Fig. 85. Inversion bridge with the fragment during first anaphase in plant no. 1.

Fig. 86. Second division bridge and fragment in plant no. 2.

Fig. 87. Showing two weak points where the dicentric bridge is likely to break.

Fig. 88. Despiralization of a lagging bivalent.

Fig. 89. Spontaneous chromosome breaks in *montana*.

Fig. 90. Spontaneous chromosome aberrations in *reflexa*; note the structurally altered chromosome, marked *X*, with subterminal primary constriction.

Fig. 91. Giant tetraploid pollen grain in *reflexa*.